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A SYSTEM OF
BACTERIOLOGY
IN RELATION TO MEDICINE

VOLUME V

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For this Volume Dr. F. C. Minett, Professor S. L. Cummins and Professor R. T. Hewlett have assisted in the editorship with regard to particular sections.

A
SYSTEM OF BACTERIOLOGY
IN RELATION TO MEDICINE

VOLUME V

BY

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CHAPTER I. GLANDERS.

BY F. C. MINETT (RESEARCH INSTITUTE IN ANIMAL PATHOLOGY, ROYAL VETERINARY COLLEGE, LONDON), WITH A SECTION BY W. BULLOCH (LONDON HOSPITAL).

(SYNONYMS: English, *farcy;* French, *morve, farcin;* German, *rotz, malleus, hautwurm.*)

Introduction.

GLANDERS is a contagious disease met with most frequently in the equine species and occasionally in man, caused by a specific micro-organism, *Bacillus mallei.* The disease is characterized by the formation of nodular lesions in certain internal organs, principally in the lungs, and by ulcerative lesions of the skin and respiratory mucous membrane. It has been usual in the past to restrict the term '*farcy*' to cases of glanders in which lesions were present on the skin.

Historical.

BY W. BULLOCH.

Under the terms μᾶλις and μηλίς certain diseases of the equine race were known in ancient times. The Latin word malleus is believed to be what we now call glanders. The views that have been held with regard to the nature of this disease have been varied and will be found in the works of Waldenburg (1869), Laboulbène (1883), and Löffler (1886). The transmissibility of glanders was proved in the eighteenth century, but denied by several authorities in France early in the nineteenth (1855; 1864–5). The particulate nature of the glanders virus was shown by Chauveau (1868), but its identification was accomplished by Löffler and Schütz (1882) in a paper published under the name of Struck, the President of the Kaiserliche Gesundheitsamt, where the work was carried out. Löffler and Schütz disclosed most of the fundamental facts regarding the bacillus of glanders (*B. mallei*). They found it in the glanders lesions of horses, and accurately described its morphology. They isolated pure cultures on blood-serum and with these pure cultures they produced typical glanders in horses. They also showed that the bacilli are pathogenic to other animals and particularly so to guinea-pigs and field mice. In these animals they described the lesions with great care, and showed the remarkable testicular reaction which may occur in guinea-pigs. This reaction was later described in detail by I. Straus (1889). Four years after Löffler's first paper with Schütz, he published another (Löffler, 1886), in which he gave a masterly account of the bacteriology of glanders and practically without an error. Little has really been added to the pure bacteriology of glanders since Löffler gave us his account. Claims for the discovery of *B. mallei* have also been made by O. Israel (1883) and by Bouchard, Capitan and Charrin (1882, 1883), but they cannot be compared with those of Löffler and Schütz, whose work was early confirmed by Kitt (1885) in animals, and by Weichselbaum (1885) in human glanders. It was apparently Kitt who first drew attention to the remarkable growth of *B. mallei* on potato. Following the lines of Koch's tuberculin an analogous substance was produced by Russian workers, especially Helmann (1891) and Kalning (1891) and by V. Babes (1891) who named it 'mallein'. The application of this substance as a diagnostic agent in glanders soon spread everywhere. In more recent years, additional diagnostic serological tests have been introduced, such as agglutination (Schnürer, 1905), and complement fixation (Schütz and Schubert, 1909, and Miessner and Trapp, 1909). Melioidosis, a disease very similar clinically and anatomically to glanders, has been described by Whitmore (1913) and by Stanton and Fletcher (1925) (see Chapter II). W. B.

Incidence of the Disease in Different Parts of the World.

Until the end of the nineteenth century, glanders was widespread among equines in all parts of the world where these animals existed in large numbers, and particularly in cities. Its full incidence in outbreaks was not realized, however, until the year 1890, when the introduction of mallein made it possible to bring to light occult cases. Thanks to the early diagnosis rendered possible by this reagent, and by serological methods, glanders may now be controlled effectively, and in the more civilized countries the disease has declined to negligible proportions or has become extinct.

In Great Britain no statistics are available up to 1870, and according to Hunting (1908), the figures cannot be considered even approximately accurate until 1881. Between 1887 and 1907, about 1,950 horses on an average were reported to be affected annually. The largest number of cases, viz. 3,001, was reported in 1892. That glanders is principally a disease of cities is shown by the fact that London and the neighbouring counties were responsible for between 80 and 95 per cent. of the annual total. In 1909 there were 533 outbreaks, involving 1,753 animals; in 1915 the number of outbreaks had declined to 50, involving 87 animals. Since 1915 the decline has been more or less gradual. In 1924 there were only two outbreaks, involving three animals, and since 1926 no cases have been reported.

In the United States of America glanders was formerly quite prevalent, but has been virtually eradicated during the last six years (Mohler).

The complete eradication of glanders from Germany has not yet been realized. Statistical records have been maintained since the year 1886. From 1886 to 1889 the annual returns show that the average number of glandered animals was 1,242. Since 1889 the number of cases has diminished with small annual fluctuations, and in 1914 only 256 cases were reported. As the result of the war the number of cases rose to 2,206 in 1915, and remained at a high level until 1920. Since 1920 a distinct fall has occurred, and in 1926 approximately 100 cases were recorded (Wehrle, 1927).

In France the statistics of the civil veterinary services show that in 1895 1,992 infected horses were slaughtered. By 1908 the number had been reduced to 398, and by 1913 had fallen to 136. During the first two years of the war the figures showed a slight tendency to rise, but by 1920 the number of cases had dropped to 96, and in 1927 18 cases only were reported in the whole of France. In the French Armies during the period of the Great War, glanders was diagnosed in 58,843 animals. Of these 20,879 represented a complete loss to the service in that 294 died of the disease and 20,585 were slaughtered.

It is impossible to arrive at an approximately accurate estimate of the incidence of the disease in human beings for the reason that owing to its comparative rarity many cases have remained unrecognized, or have been confused with other conditions. During the period 1891 to 1897, 27 deaths were certified to have been due to glanders. M'Fadyean (1905) observed from the reports of the Registrar General that in Great Britain about four people died of glanders annually—during a period of 20 years (1883–1902) 78 deaths were certified to have been due to this cause. Broadly, the incidence in man is proportional to the incidence in equines. Glanders is an occupational disease in that it chiefly occurs among persons who have to do with horses. Such operations as the administration of medicinal agents by the mouth are likely to be particularly dangerous. In this respect, the living horse forms a more dangerous source of infection for man than the dead animal, owing to the fact that glanders bacilli are confined to the lesions and discharges and are never numerous in the blood. M'Fadyean ascertained by enquiry of a large horse-slaughtering firm in London that no cases of human glanders had occurred among employees who over a period of 12 years had handled annually between 800 and 2,000 glandered carcasses. Robins (1906) collected the available records of 156 human cases in which a diagnosis of glanders had been made. In 61 of these the diagnosis was based on the isolation of *B. mallei* or on the production of characteristic lesions in an inoculated donkey, horse or male guinea-pig. In 76 cases the diagnosis was regarded as 'probably correct', but could not be taken as proved since experimental proof was wanting. In 99 of the 156 cases, there was a history of contact with glandered equines, and in 4 other cases the patients were horse slaughterers.

Morphology of the Causal Organism.

In the lesions and discharges *B. mallei* appears as a straight or slightly curved rod with rounded ends, 2·0 to 5·0μ long and 0·5μ broad. In point of size it has been frequently compared with the tubercle bacillus, though on the average it is somewhat broader than this organism. In smears of pus many of the bacilli stain irregularly and present a characteristic beaded appearance, the unstained portions possibly representing areas of degeneration. These unstained areas certainly cannot be taken to represent spores, as was thought by some earlier observers. *B. mallei* does not form spores, and it does not possess a demonstrable capsule.

In smears of pus the majority of the bacilli are extracellular, but a small number can usually be found within leucocytes. Apart from this there is no special arrangement, the organisms lying in disorderly fashion among the cells and cell debris. In sections from acute lesions of the internal organs treated with a simple stain it is often difficult to identify the bacilli, owing to the fact that they are beaded and that they lie amid disrupted masses of chromatin. Added to this is the fact that even in acute lesions bacilli are not very numerous. For instance, in smears of farcy pus from horses it may be necessary to search several fields before a single organism is found, and in chronic lesions such as old pulmonary nodules in the horse it is unusual to find any bacilli microscopically.

In young cultures on solid media the rods tend to be shorter and fairly uniform in size. In older cultures there is much more pleomorphism than in the case of bacilli from lesions. An extreme example of this is seen in the surface scum which forms on broth. Here the organism tends to grow out into filaments, 12μ or more in length, which frequently exhibit true branching and show small terminal swellings. The filaments usually stain rather lightly with simple stains, and show at intervals inset particles which take the colour more intensely.

Duval and White (1907) believed that the morphology of the organism could be correlated with the toxicity of the strain. With diminishing toxicity, such as may result from subcultivation, they noted a change from the short plump appearance to long thin rods with clear spaces at irregular intervals along the body.

Young broth cultures of *B. mallei* examined in hanging drop show no evidence of true motility. Stanton and Fletcher (1925) subcultivated five strains in liquid media daily for three weeks without observing motile forms.

Staining.

Numerous methods have been recommended for staining the glanders bacillus. The stain originally recommended by Löffler was his alkaline solution of methylene blue, followed by differentiation with 1·0 per cent. acetic acid for a few seconds. Simple staining solutions, however, such as methylene blue or fuchsin are quite suitable for displaying the bacilli in smears of pus or from cultures. Following fixation by heat or by alcohol, smears of pus may be treated for a few seconds with 1·0 per cent. acetic acid after the staining solution has been washed off, but in reality there is less danger of decolorizing the bacilli than is commonly supposed. M'Fadyean (1904) found that they were not completely decolorized after treatment for a few seconds with 10·0 per cent. solution of acetic acid.

Good results may also be obtained with carbol thionin, followed by differentiation with aniline oil, though if the staining solution is of suitable strength differentiation is unnecessary. On the whole *B. mallei* does not stain intensely. Bacilli from young cultures take the stain fairly uniformly, while those from older cultures and from tissues have a beaded appearance. The granules contained within the substance of the bacillus are occasionally situated close to the extremities, and with methylene blue the staining of the granules may be metachromatic. The bacillus fails to retain the stain by the method of Gram or its modifications (Gram-Weigert, Gram-Nicolle, Claudius).

For the reasons mentioned in the last section, the satisfactory demonstration of the organism in sections is not always a simple matter. M'Fadyean (1904) recommends the following method for sections of tissues which have been fixed in alcohol. Stain for 30 minutes with Löffler's alkaline methylene blue, with Kühne's carbol blue or with 1·0 per cent. methylene blue in 10·0 per cent. alcohol; wash; treat with 4·0 per cent. acetic acid for a few seconds; wash; mordant for 15 minutes with a saturated solution of tannin; wash thoroughly; counterstain with 1·0 per cent. acid fuchsin for 30 seconds; dehydrate with alcohol as rapidly as possible; clear with cedar wood oil, and mount in balsam. The tissues have for the most part a faint red tinge, while the bacilli are light blue.

Other somewhat more cumbersome methods have been recommended for sections, but it is doubtful whether they have any special advantage.

Cultivation.

Multiplication of the organism under aerobic conditions occurs in practically all the common culture media at an optimum temperature of about 37° C. No growth takes place below 20° C. or above 42° C., but fair growths are still obtainable at 32° C. Growth is possible within a comparatively wide range of reaction, and the abundance is not diminished by slight acidity or slight alkalinity of the medium, in fact slight acidity is said to be favourable to development. The two outstanding characteristics of cultures are their sliminess and their tendency to develop a brownish coloration with age.

Broth cultures during the first two or three days become moderately and uniformly turbid. In typical cases in the course of a week spots of white or very faintly yellow slimy surface growth appear, as well as a variable amount of slimy bottom growth. The amount increases up to a point with continued incubation, and the medium becomes darker in colour. Cultures possess a faint characteristic odour which cannot be easily described.

Growth on *agar* and *glycerin agar* are similar in being translucent and slimy or tenacious, and in becoming yellowish or slightly brownish with age and at the same time more opaque. On agar to which 3 or 4 per cent. glycerin has been added, development is more abundant than on plain agar. On the other hand, the addition of glucose to agar hinders growth. In stab agar cultures growth is confined to the needle track.

Solid horse- or *sheep-serum* was first recommended by Löffler and Schütz (1882) as a highly suitable medium. On the second or third day surface colonies appear in the form of faintly yellow, transparent droplets, which after a week's incubation become milky white.

Potato. Great interest attaches to this medium on account of the characteristic nature of the growth thereon. When it first appears, it is translucent and possesses a slightly yellowish or amber tint ; young colonies have been accurately described as resembling drops of clear honey. With continued incubation it becomes darker, passing in the course of two or three weeks to reddish-brown or dark fawn, and finally in some cases to a chocolate tint. The medium itself develops a pale or dark green coloration, which is especially noticeable in the neighbourhood of the growth. Young growths are slimy and with age become more tenacious. An important distinctive point to be noted is that, though colonies may coalesce by increase of size, they exhibit no tendency to spread over the medium. While the colour changes just described may be regarded as characteristic, Wladimiroff (1913, p. 1087) among others has insisted upon the variability in this respect which may be shown by the same or different strains of *B. mallei.*

In *gelatin* stab cultures at a temperature of 22° C., slight growth only occurs, without liquefaction. In the case of five strains examined by Stanton and Fletcher (1925), after growth at 37° C. for 26 to 40 days, the medium remained liquid on being cooled.

Lührs (1927, p. 13) found that the glanders bacillus is capable of giving luxuriant surface growths on a neutral, albumose-free fluid medium of the following composition : monosodium phosphate 15·0 gm., monopotassium phosphate 20·0 gm., magnesium sulphate 3·0 gm., magnesium citrate 12·5 gm., asparagin 25·0 gm., glycerin 100·0 gm., distilled water to 5,000 c.cm. The addition of small amounts of iron (5·0 to 10·0 mgm. ferro-ammonium sulphate per litre) are said to be extremely favourable to growth.

Isolation of B. mallei *from the tissues. Selective media.* When using ordinary methods, it is particularly desirable to attempt isolation from material uncontaminated with other organisms. Not only are glanders bacilli likely to be present in comparatively small numbers, but also they are liable to be overgrown owing to their slow multiplication in primary culture. Several slopes of solid horse-serum and of potato should be richly sown with the infective material, the tubes capped and incubated at 37° C. In the case of potato, the material should be thoroughly applied to the surface. No growth is to be expected in less than 48 hours, and with potato three days frequently elapse before growth is visible for the first time in the form of isolated colonies. After a few subcultures development becomes more rapid, and continuous growth may then be obtained on the common media within 24 hours.

Viability of cultures. The glanders bacillus in culture is not highly resistant. Cultures on solid media which have been incubated for 2 or 3 days at 37° C. and then removed to room temperature may show no surviving organisms at the end of two months. Cultures left at 37° C. for a month may show no surviving organisms on subcultivation.

Maintenance in artificial culture. For the purpose of maintaining living cultures for long periods without the necessity of subculturing, many of the methods found to be suitable for delicate organisms would no doubt give satisfactory results. According to Adil Bey, quoted by M. Nicolle (1906[1]), cultures can be preserved in normal horse-serum which has been diluted with three parts of distilled water before being autoclaved. Agar growths are added to this medium to form a thick emulsion, and the tubes sealed and stored in the cold. After a year active subcultures could be readily obtained.

Cultural Variants.

Blaimschein (1917) by plating on glycerin agar one-month-old glycerin-broth cultures of *B. mallei* noted with 10 strains the development of three different types of colonies. Type 1 colonies representing the normal form were mucoid in character, circular in outline, and possessed smooth surfaces. In colour they were dull-white, or bluish-grey, becoming slightly yellow with age. Type 2 colonies on glycerin agar were distinguished by their smaller size and deep amber-yellow colour, by their thickened edges and their irregular outlines. Microscopically the centres of the colonies were seen to be depressed and wrinkled. Colonies were dry and so coherent that they could readily be lifted whole from the surface of the medium. Type 3 colonies were similar macroscopically to Type 1 but were thicker and when magnified were seen to be less homogeneous and to possess a more or less coarsely wrinkled surface.

Type 2 variation was closely associated with the capacity of a strain to produce filaments, since the variation was especially liable to occur with strains which formed long threads in broth. Growth on potato was dry and granular, but subcultivation on this medium as well as in broth produced a gradual reversion to Type 1.

No alteration of pathogenicity or loss of power to produce efficient mallein was shown to accompany the change of type, though the experiments on these points cannot be said to have been very searching.

Biochemical Reactions.

With the exception of glucose *B. mallei* has no action on commonly employed test substances. Even with glucose fermentation is inconstant and varies with the strain of organism and with the medium employed. In glucose peptone water some strains produce acid after 10 to 15 days at 37° C. Slight acid production sometimes occurs in litmus milk with coagulation by the tenth day and decolorization of the litmus in the lower parts of the medium. No indole is formed in peptone media, and nitrates are not reduced. Bile has no appreciable solvent action.

Products of Growth.

Endotoxins.

That the glanders bacillus produces toxic substances in culture has been known since the work of A. Babes in 1890 (V. Babes, 1894). The production of such substances in the body accounts for the pyrexia and emaciation seen in natural cases of the disease. When introduced into healthy animals, such extracts are capable in certain circumstances of causing great constitutional disturbance. The dead bodies of the bacilli alone are also capable of exciting morbid change, and acute fatal infections in susceptible animals have been produced with bacilli killed by heat (60° C.) or by alcohol. Thus Cantacuzène and Riegler (1907) treated bacilli from agar cultures with alcohol, and after centrifuging and evaporation obtained a dry powder, 100 to 150 mgm. of which by the mouth was sufficient to kill both young and adult guinea-pigs. On post-mortem examination the dead bacilli could be detected in the mesenteric glands and spleen, and in the lungs, where broncho-pneumonia was a constant lesion. Smaller doses (25 to 50 mgm.) caused cachexia and death within 2 to 4 weeks. Still smaller amounts (5 to 15 mgm.) produced no immediate ill-effect, but the animals were sensitized so that severe disturbance followed a second feeding with similar small doses. The sensitive state was observed to last for about three months. Products toxic in varying degree for guinea-pigs had been obtained previously by M. Nicolle (1906^2) by treating bacilli with equal parts of alcohol and ether, or with chloroform. Turro (1908) dissolved glanders bacilli in 0·5 per cent. sodium hydrate, precipitated the alkaline extract with alcohol, and by drying the precipitate obtained a powder, which in a dose of 1·0 mgm. produced œdema, and sometimes death, on subcutaneous inoculation in guinea-pigs.

Mallein.

The preparation now known by this name consists of the heat-stable products which are formed in glycerin broth cultures by the growth of *B. mallei*, and which are extracted from the bacterial mass during the heating process.

Mallein has been widely used for the diagnosis of the disease in equines, and has been tried for the treatment of the disease in man. Comparatively large amounts of mallein may be injected into healthy animals by the subcutaneous route without effect, whereas very small amounts set up severe disturbance in animals affected with glanders. The manner of using mallein for diagnosis is discussed below (pp. 36 et seq.).

The reagent was first prepared by Helmann and Kalning in 1890 by extracting old potato cultures for a few days at 37° C. in a mixture of equal parts of glycerin and water, and then steaming and filtering. Foth (1892) recommended purification of the active substance by alcohol precipitation. Glycerin broth cultures were evaporated to one-tenth of their volume, filtered and the filtrate treated with 20 volumes of absolute alcohol, the precipitate being collected and dried *in vacuo*.

A. Babes (1892) precipitated filtrates of broth or potato cultures by means of a mixture of equal parts of alcohol and ether. The precipitate when dried was known by the name of ' morvin '.

As previously stated, at the present time mallein is usually prepared from broth cultures. It is generally assumed that the strain used for sowing the cultures should be virulent, and that the efficiency of the mallein is likely to be impaired if the virulence of the seed culture has diminished as the result of prolonged cultivation on artificial media. There is some field evidence which goes to show that this assumption is correct, though experimental proof is lacking. The usual method of preparation is to cultivate highly virulent bacilli in 3 to 5 per cent. glycerin peptone broth for a month. The culture is then sterilized by steaming or autoclaving, filtered through paper and the filtrate evaporated to one-tenth of the original volume. The final product, known as crude mallein, is a dark-brown, syrupy liquid with a characteristic odour, which retains its activity for months if stored in a dark, cool place.

Mallein is also frequently employed without preliminary evaporation —in the so-called dilute form—and phenol is added as a preservative up to 0·5 per cent. concentration. This dilute product also retains its properties for months or years if kept in a dark, cool place. It has been considered by some that the potency of mallein may be increased by incubating the cultures for longer periods than a month, e.g. to 8 or 12 months, but there is no real evidence that this is so. On the assumption that the activity of mallein can be judged by the results of complement-fixation tests, Vehse (1920) concluded that no advantage was gained by incubating cultures beyond 25 days.

Standardization of mallein. While mallein prepared from glycerin broth cultures of a virulent strain can usually be relied upon for diagnostic purposes, a satisfactory method for standardizing the reagent is desirable. Schnürer (1910) was the first to attempt this by injecting glandered horses intradermally with diminishing amounts of the mallein under test. The potency of the reagent was judged after 24 hours from the size of the local swelling which resulted. Reactions may be produced by crude fluid mallein in amounts as low as 0·0005 gm. or by Foth's dry mallein in one-tenth of this amount.

Schreiber and Stickdorn (1915) believed that the complement reaction was capable of affording reliable quantitative information as to the diagnostic value of mallein. By means of a suitable antiserum, different preparations of mallein could be compared with a standard sample of dry mallein, and the values expressed as antigenic units. The unit was defined as the minimal amount of mallein which in the conditions of the test caused complete inhibition of hæmolysis. A sample of crude liquid mallein was found to contain 200 units to 1·0 c.cm. and a sample of dry mallein 400 units in 0·03 gm. Samples of liquid and of dry mallein of the potency stated were found to be capable of producing eye reactions in glandered horses, and were considered by the authors to be suitable for field use. Watson and Heath (1922) also consider the complement-fixation reaction to be a useful means of standardizing mallein. These authors prepared an antimallein serum from horses by which the complement-fixing power of different specimens of mallein could be compared. A precipitin test which had been previously recommended was not found to give clear-cut results. According to Lührs (1927, p. 56), the results of the complement-fixation reactions with mallein antigens are not always clear owing to the occurrence of zone phenomena.

Giese and Krüger (1924) concluded that mallein could be standardized by determining the smallest quantity capable of killing infected male guinea-pigs within 24 hours.

The problem of mallein standardization is no doubt similar to that of standardizing tuberculin. This question has been reviewed recently by Calmette and de Potter (1926), who conclude that neither precipitation nor complement tests reflect the true reacting power of tuberculin. Methods involving a determination of the minimal fatal dose of tuberculin for tuberculous guinea-pigs are expensive and merely indicate the toxicity of the product. The final conclusion of these authors is that the most satisfactory method of standardizing tuberculin is by the use of intradermal tests in sensitized animals (tuberculous guinea-pigs and oxen), though the results can only be held to apply to the species of animal under experiment. If these conclusions are accepted, it is apparent that the value of mallein should be judged by intradermal tests on glandered guinea-pigs, and that the results should be checked if possible on glandered horses according to the method originally proposed by Schnürer.

Serological Reactions.

In natural cases of glanders in horse and man the blood-serum acquires specific immune properties. Experimentally in horses and in small laboratory animals, such as rabbits, similar changes in the serum may be produced by the injection of either living or dead bacilli.

Agglutinins. The presence of specific agglutinins in horse-serum was first noted in 1896 by M'Fadyean, and in the following year by Foulerton in human serum. Schütz and Miessner (1905) found that in experimentally infected horses agglutinins appear in the blood after 4 to 8 days, and reach their maximum titre (1,000 to 2,000) by about the ninth or eleventh day. The titre begins to decline a month after infection, and subsequently there are often considerable fluctuations in the agglutinating power. The serum of normal horses also agglutinates the bacilli, but usually in a dilution of less than 1 in 500. Normal human-serum agglutinates the organism up to about 1 in 100 dilution ; during infection the titre may rise to 1 in 1,000 or 1 in 2,000.

Precipitins. Pfeiler (1909) among other observers detected precipitins in the blood of horses 4 or 5 days after experimental inoculation. Although the titre reached was not so high, it ran approximately parallel to that of agglutinin, declining as the disease passed into the chronic stage. Precipitins have also been detected in the serum of natural cases in horses.

Complement-fixing bodies. These have been detected in the serum of human cases of glanders, and their presence in natural and experimental cases of the disease in horses is well established since the work of Schütz and Schubert (1909) and of Miessner and Trapp (1909). Experimentally it has been found that in the horse the specific properties of the serum are present a few days after infection, e.g. 5 to 7 days in two cases reported by Schütz and Schubert, and with periodic fluctuations may be detected for long periods in cases which have become chronic. In natural cases the reaction frequently does not become positive before the end of the second week, and shortly afterwards the serum may be expected to give complete fixation in amounts of 0·1 c.cm. or less. It is very rare to find the serum of healthy horses giving fixation in 0·1 c.cm. to 0·2 c.cm. amounts, but it is important to note that the serum of healthy horses which have been injected with mallein reacts positively to the test. Further details regarding serum reactions from the point of view of diagnosis are given below (p. 41).

Serological Races.

A good many observers have pointed out that strains of *B. mallei* vary in agglutinability or in their antigenic potentiality in complement-fixation reactions. Stanton and Fletcher (1925) in the course of their work on melioidosis showed that there are distinct serological races of *B. mallei*, some of which are closely related to *B. whitmori*, the causal organism of melioidosis. These authors examined five old laboratory strains of *B. mallei.* Of these strains two had been originally isolated from horses in India and in Java respectively, and the remainder from horses in England and in Egypt. By agglutination, agglutinin absorption and by complement fixation, the last three strains were found to fall into one group, which was sharply differentiated from the Indian and Javan strains, and these in turn were closely allied to fourteen strains of *B. whitmori.* Stanton and Fletcher concluded that a group of organisms differing from each other serologically has been included under the name *B. mallei*, and that *B. whitmori* is a member of this group.

Organisms Resembling B. mallei.

A certain number of organisms have been described which resemble the glanders bacillus in one or more important respects, such as morphology, staining, cultural characters or pathogenic effects in male guinea-pigs. So far as pathogenicity is concerned, various types of micro-organisms are capable of producing in male guinea-pigs effects which were at one time regarded as diagnostic of *B. mallei.* Apart from these, the literature contains a few references to bacterial types which are more or less closely related to *B. mallei.*

Malassez and Vignal isolated from cases of pseudo-tuberculosis in rodents a Gram-negative, polymorphic streptobacillus, which was shown by Basset (1907) to produce a Straus reaction in guinea-pigs.

V. Babes (1891) applied the term 'pseudoglanders' to a diseased condition in horses which bore some clinical resemblance to glanders. From one case he isolated a Gram-negative bacillus, morphologically similar to *B. mallei* and giving a somewhat similar growth on potato. The organism differed from the true glanders bacillus in being strongly pathogenic for rabbits and non-pathogenic for guinea-pigs and for a horse.

Martini (1911) isolated from a muscular abscess in a man a Gram-negative bacillus, showing polar staining with Löffler's methylene blue, growing like *B. mallei* on potato, and producing a Straus reaction in one out of two inoculated guinea-pigs. According to the author, the organism was essentially different from the glanders bacillus in one important respect only, viz. alkali production in milk within 24 hours. From the description, however, it is noted that acid was produced from glucose, maltose and lactose.

Bulloch and Twort (1905) described a Gram-negative bacillus, resembling *B. mallei* morphologically and growing like it on potato, which was isolated from suppurative lesions in a woman. The organism was incapable of producing a Straus reaction in guinea-pigs, but in three cases out of seven death occurred within 24 hours, and the same organism could be recovered *post mortem*.

Reverdin and Grumbach (1924) described a Gram-negative 'paraglanders' bacillus isolated from a case of chronic pyæmia in man. The patient's serum gave strongly positive complement fixation with three different glanders (?) antigens. Growths on potato resembled glanders in being ' honey-like, transparent and viscous ', but the colour tended to become ' bright yellow ' with age and was not regarded as typical. Of ' several ' male guinea-pigs inoculated, one alone showed a gelatinous exudate on the tunica vaginalis, while in all cases there was congestion of the abdominal vessels and septicæmia, but no actual nodule formation in the internal organs. The bacillus was also pathogenic for mice and rats. Mice usually died within 24 hours with septicæmia ; rats died in a month and the bacillus could be recovered from the blood and spleen.

Whitmore (1913) isolated from cases of ' melioidosis ' in man a Gram-negative organism, which was shown later to belong to the *mallei* group. (See Chapter II.)

Pathogenic Action.

Susceptibility.

Glanders has been observed to occur naturally in equines (horse, ass and mule), in man, in certain wild felines (lions, tigers, leopards) and in wolves. Among equines, the ass is so highly susceptible that chronic glanders in this animal is almost unknown (Hunting, 1908). The camel is also susceptible. Sheep and goats can be infected, though their susceptibility is not great; goats, however, are more susceptible than sheep and there is evidence to show that occasionally they have been infected naturally. Cattle and swine are practically immune.

Animals which have been used for experimental purposes include guinea-pigs, rabbits, cats, dogs and field mice, though the susceptibility of these species is far from being equal. Thus rabbits are more resistant than guinea-pigs and on the whole dogs are more resistant than cats. The susceptibility of mice varies with the species; white mice are far more resistant than field mice. White rats are immune, and birds have never been successfully infected.

Experimental Infection.

By means of infective discharges or virulent cultures the disease may be set up in susceptible animals by practically any route of inoculation. Thus in the *horse* the experiments of Nocard (1894, 1896) showed that, by feeding horses with small amounts of virulent culture mixed with the drinking-water or enclosed in pieces of bread or carrot, evidence of disease could be produced within eight days. In some animals lesions were confined to the thoracic cavity, in others they developed also in the submaxillary glands and on the mucous membrane of the nasal septum and turbinate bones. Schütz (1898) by feeding horses with considerably larger amounts of culture enclosed in gelatin capsules produced lesions not only in the lungs, but also in the liver and mesenteric glands. Experiments on feeding of equines have also been made by M'Fadyean (1904), Miessner and Trapp (1909) and Hutyra among others. It has been demonstrated that the distribution of lesions in the horse after feeding virulent material depends to a large extent upon the quantity fed. Hutyra (1907) found that after feeding small amounts of culture, a fever reaction invariably followed 3 to 5 days later, and that at this period bacilli could be demonstrated in the circulating blood. Inhalation experiments, though in some ways unsatisfactory, sometimes furnished evidence (Hutyra, 1907) which was unfavourable to the view that inhalation is the usual method of infection in nature, inasmuch as the inhaled bacilli produced local lesions in the nasal passages, lesions which are frequently absent from natural cases. Horses may be infected easily by the subcutaneous route; Marxer (1919), for example, observed fatal effects after inoculation of $\frac{1}{250,000}$ loopful of culture.

In the *ass* infection may be readily transmitted by cutaneous inoculation, and this method was frequently used by earlier observers for purposes of diagnosis. If the material used is virulent, acute glanders follows with death in two weeks or less. At one time cutaneous inoculation was also frequently resorted to in the *cat* and in the *dog* for the same purpose.

The importance of the *guinea-pig* as a test animal in glanders was pointed out by Löffler (1886). This animal may be infected by any method of inoculation, including cutaneous scarification. Löffler noted the tendency of the causal organism to localize in the neighbourhood of the testis, though the nature of this reaction was first accurately described in 1889 by I. Straus. The experimental disease in the guinea-pig has also been studied by M. Nicolle (1906[1]). After intraperitoneal inoculation the organism localizes primarily in the serosa covering the muscle which controls the movements of the testis, hence the first sign noted in the infected animal is a flaccid condition of this organ. The important role played by the anatomical structure of the genital region in the male guinea-pig in favouring localization of *B. mallei* is shown by the fact that castration involving complete removal of the testicular muscle increases the resistance to infection by the intraperitoneal route. In the male guinea-pig two forms of the disease can be distinguished, depending upon the quantity and virulence of the bacilli injected. If a sufficient dose of virulent bacilli is injected, the characteristic Straus reaction appears (see p. 35); if smaller amounts are given, a more benign or ectopic form of disease is produced, which is frequently followed by recovery. This form is characterized by the persistence of the testis in the abdomen close to the inguinal ring, where it is held in place by lesions involving the testicular muscle.

The *rabbit* possesses in general a low susceptibility, and the subcutaneous route is rather uncertain unless large amounts of infective material are used. Large doses intravenously sometimes produce death in a few days (Löffler, 1886) and the organism can be adapted to the rabbit by passage. Löffler (1886) pointed out the high susceptibility of field mice. The same observer, as well as V. Babes (1891), showed that though white mice are resistant they can be infected with pure cultures, and the facts relating to these animals have been confirmed by M. Nicolle (1906[3]), and more recently by Zabolotny (1926). After inoculation of white mice with freshly isolated culture, a proportion die within 72 hours without marked changes in the organs. After inoculation with pus, death is delayed to the fifth or sixth week, when lesions may be seen in the spleen and liver. For diagnostic purposes the animals may be killed after 10 to 13 days.

Renault and Bouley (1840) showed that *sheep* could be infected, and it has been demonstrated that by cutaneous scarification the disease can be transmitted through a series of sheep and finally reinoculated to the ass.

Inoculation of large amounts of virulent culture subcutaneously in the *ox* produces local abscess formation, but as a rule from a single inoculation no further ill-effects ensue. Toxic symptoms and fatal effects have been produced, however, in a proportion of animals in experiments in which the inoculation of virulent cultures has been repeated.

Natural Infection.

The feeding experiments of Nocard, Schütz and Miessner shed light on the method of natural infection in the horse. These experiments indicated that abdominal lesions are usually absent, unless fairly considerable amounts of infective material are given, amounts far more than the animal would be likely to acquire in nature. Since in a considerable proportion of natural cases of glanders lesions are confined to the thoracic cavity, it is highly probable that the usual method of natural infection in these animals is by the ingestion of food or water contaminated with virulent secretions. More rarely equines have been infected by way of the skin through the medium of the harness.

The consumption of raw flesh from glandered horses has resulted occasionally in the infection of cats and of wild mammals, such as lions and tigers, kept in captivity.

In man the commonest way in which the disease is acquired is undoubtedly by the cutaneous route, though in some instances infection has arisen from swallowing of infective material derived from equines or from artificial cultures. According to Robins (1906) infection from man to man is less rare than is commonly supposed ; in his series of 156 cases, of which the majority were authentic, 15 such instances were met with.

Morbid Anatomy.

In the *horse* the most characteristic lesions are found in the lungs, and in apparently healthy animals which have been slaughtered on account of a reaction to mallein, they are frequently the only lesions which can be discovered during an ordinary post-mortem examination. Usually they take the form of firm nodules, embedded in the otherwise healthy tissue or visible from the pleural surface. They vary in size from that of a split pea to that of a hazel nut, though occasionally the area involved reaches the size of a small orange. While they may be present in any part of the lung, the number is very variable—from half a dozen to several hundreds—but the distribution is never miliary as in generalized tuberculosis.

The appearance of the glanders nodule in section depends upon its age. Very recent nodules are dark-red throughout, but the central portion quickly takes on a greyish, translucent aspect. Later the central area increases in extent, becomes whitish and more opaque, and is seen to be separated from the normal lung tissue by a zone of congestion. In the final stage, the nodule is surrounded by a fibrous capsule, and its centre is crumbling and slightly gritty owing to deposition of calcium salts. In rare cases the change in the nodule is of a fibroid nature.

If the disease process is very acute, it may not be confined to distinct nodules, but may involve larger areas of lung tissue and thus be more lobar in distribution. In such cases the affected areas appear dark-red and œdematous, with inset yellowish-white or greyish foci. In later stages old pneumonic areas containing yellowish-white, dry and caseous masses may be found in the lung tissue.

Nodular lesions in lymphatic glands are similar in general appearance to those occurring in the lung tissue, but in acute cases the glands may be simply swollen and congested. When the disease is advanced, lesions are frequently present on the mucous membrane of the respiratory tract, e.g. of the larynx and trachea, as well as on the septum nasi or turbinate bones. In acute cases the membrane is merely intensely congested, usually, however, there is ulceration of varying degree, and, in the case of the septum nasi, this may lead to perforation. Lesions in the nasal cavity are always accompanied by a discharge from the nose and by swelling of the submaxillary lymphatic glands.

Abdominal lesions are usually found in the spleen and liver ; in general characters they resemble the lesions found in the lungs. Lesions of the skin take the form of swellings (so-called ' farcy buds ') about the size of walnuts. They are usually situated at intervals along the course of the lymphatic vessels, the walls of which are greatly thickened. The farcy swellings break down into crater-like ulcers and discharge pus of a characteristic oily nature. Finally, in advanced cases a frequent lesion is elephantiasis of a hind limb.

Pathology in Equines.

Glanders bacilli which are ingested in small amounts are believed to make their way to the abdominal lymphatic glands and thence to the lungs, where they produce the characteristic lesions. In many cases in horses the lesions remain latent in the internal organs for months or years without any symptoms to indicate the existence of disease beyond occasional coughing, difficulty in respiration, intermittent rises of temperature and loss of condition. Frequently the disease does not progress beyond this point ; the bacilli are destroyed locally and the lesions become encapsuled. At any time, however, before the bacilli are completely destroyed, external manifestations may make their appearance. When bacilli are ingested in larger amounts or over a longer period, a sufficient number may be arrested in the mesenteric lymphatic glands and liver to produce macroscopic lesions. In the more severe cases of this type, bacilli may be detected by cultural methods in the blood, through the medium of which they are distributed to the skin and other parts of the body. In chronic cases the blood is apparently sterile, and infectivity is confined to the lesions and discharges.

Bacilli distributed by the blood are liable to be arrested at certain points in the skin and mucous membrane of the respiratory tract, where they produce lesions. Skin lesions are particularly liable to arise in situations exposed to traumatism, such as the legs. Lesions may remain present on the skin for long periods, since in spite of a tendency to heal, abscesses are liable to appear in other parts accompanied by morbid changes in the regional lymphatics. Lesions of the respiratory membrane also display a tendency to heal, the ulcers being replaced by scar tissue. Cases of farcy are accompanied by great weakness and wasting, and have been followed by recovery in but few instances.

Bacilli may be excreted from the body not only by way of the skin but also by way of the kidneys, though lesions in these organs are not common.

Bacilli introduced by way of the skin (subcutaneously or cutaneously) first produce local effects. After a few days a painful œdematous swelling develops, which is followed by abscess formation and ulceration. From this point the bacilli spread principally by the lymphatic vessels, in which they set up severe disturbance. Ultimately lesions in the lungs are produced with extension to the nose and other parts, just as may occur in natural cases. Large amounts of infective material given subcutaneously have acute and fatal effects.

In glanders in the ass the infection is almost invariably an acute one, in which clinical lesions rapidly make their appearance, with death 8 to 20 days later. The disease in the mule is also as a rule far more acute than in the horse and death after 3 or 4 weeks is the usual sequel.

Pathology in Man.

In cases in which infection is acquired by way of the skin, usually of the hand or arm, a nodular swelling develops in 2 to 6 days, with infiltration of the surrounding tissue. The part then takes on malignant characters, breaking down to an ulcer with sharply defined borders. The discharge which appears may be at first thin and watery, later it becomes thick and ropy and tinged with blood. In general, lesions strongly resist treatment ; partial healing often takes place, usually with extensive loss of tissue and cicatricial changes. Frequently bony tissue in the neighbourhood is attacked, with necrosis and sinus formation as a result. In the majority of cases the disease does not long remain localized, severe constitutional disturbance (fever and acute muscular pain) indicating extension to internal organs, and lesions appear on the skin or in the subcutaneous tissue in remote parts of the body. Suppurative changes in the joint cavities and in the muscular masses are also observed, and the clinical picture then resembles that of a malignant pyæmia.

The disease in man resembles that in the horse in that localization to a greater or smaller extent takes place in the lungs. V. Babes (1894) encountered small sclerosed or calcified nodules in the lungs of persons who had been employed among horses, and who had died of affections other than glanders. He believed these persons to have been suffering from latent glanders, and in one case in which caseous masses were present in the lungs, he was able to isolate an organism having the ' greatest analogy ' with *B. mallei*. According to Robins (1906) the lesions in the lung tissue are usually no larger than peas, and in recent cases are surrounded by zones of congestion. Sometimes they may soften and coalesce, forming large necrotic areas or definite abscesses. Pleurisy has also been noted. Ulceration of the nasal mucosa with accompanying nasal discharge has been observed many times and in some instances this appeared to be the primary change. Lesions in this region may be followed by perforation of the septum or by partial destruction of the turbinate bones or of the palate, with extension of the disease process to the bones of the skull. Otitis media has also been observed.

The course of the disease is very variable, both in its manifestations and in its duration. If systemic invasion is rapid, the disease is likely to end in death of the patient from exhaustion within 2 to 6 weeks. On the other hand, an attack may last for years and show acute recurrences with periods of apparent recovery up to 1 to 5 years. Several cases are on record in which the attack lasted for 4 to 6 years, and in one case for 11 years before a diagnosis was established. Other cases are known which commenced without primary local change, and in these ingestion was the probable method of infection. In some instances the first symptoms, hoarseness, cough and difficult breathing pointed to the respiratory tract as the site of infection. In one such instance the attack began as a chronic pneumonia and the true nature of the condition was not realized until skin and nasal lesions appeared two years later. This case ended fatally after 4 or 5 years.

When the infection is acute, the patient usually dies. In chronic cases it is difficult to form a satisfactory estimate of the mortality, since recovery may be merely apparent. No doubt immediate surgical treatment of lesions as they arise has led to many recoveries.

Analysis of Pathogenic Action of B. mallei.

Virulence. This factor plays an important part in the pathology of the disease and in a given species determines the severity of the attack. The initial virulence of a culture has been considered to be dependent to some extent upon the species of animal from which it is derived. According to Bulloch and Twort (1905) the virulence of bacilli from both acute and chronic human cases is likely to be especially high, small amounts (1·0 mgm.) of young culture intraperitoneally killing guinea-pigs on the third day.

Virulence is a characteristic of *B. mallei* which is to some extent under control. With cultivation outside the body it slowly diminishes and according to M. Nicolle this decrease (1906[1]) is sometimes accompanied by a diminution in abundance of growth and an increase in fragility. The rate of virulence loss is dependent to some extent upon the particular medium used. For example, the loss is more rapid on agar made from potato broth than on agar made according to the formula of L. Martin. According to M. Nicolle (1906[1]) the virulence of some strains may not be entirely lost after months or even years existence on artificial media. Stanton and Fletcher (1925) examined three strains which had been maintained in artificial culture for 'long' periods. Two of the three strains inoculated subcutaneously in guinea-pigs produced local abscess formation only, which was followed by recovery, and one of the two strains failed to produce glanders when inoculated into a horse. The third strain was incapable of producing even local changes in guinea-pigs or in a horse on subcutaneous inoculation.

The general standard of virulence may be increased by passage through animals. Among the older methods recommended were passage through young cats, squirrels and asses. At the present day for purposes of mallein preparation the virulence of the organism is increased by intravenous passage in rabbits, during which the disease acquires septicæmic characters, or by the intraperitoneal inoculation of male guinea-pigs. By injecting guinea-pigs in serial passage with both living bacilli and with the toxic material obtained by dissolving glycerin agar culture in 0·5 per cent. sodium hydrate, Turro (1908) was able to increase the virulence to the point where the disease became septicæmic with death resulting in 16 to 20 hours. The organism also gains in virulence on successive passage through the white mouse (M. Nicolle, 1906[3]). An increase of virulence for one species, however, may be accompanied by a decrease of virulence for another species. Thus, starting with a virulent strain from equines, M. Nicolle (1906[3]) by means of guinea-pig passage was able to increase the virulence for guinea-pigs, but at the same time the virulence for the rabbit and the white mouse was lowered.

Endotoxic action. In conformity with the usually accepted doctrine the virulence or invasive capacity of the glanders bacillus may be looked upon as primarily dependent upon its power to elaborate toxic substances within the body. Strains which have lost their infecting power to a large extent by subcultivation have also lost much of their toxicity. At any rate, animals injected with such strains do not suffer from the severe general disturbance which is believed to be due to the liberation of endotoxins. In agreement with this conception, Turro (1908) found that the toxicity of agar cultures dissolved in sodium hydrate varied directly with the initial virulence of the culture. Moreover, in preparing an efficient mallein—which may be regarded as a product containing the endotoxins of the organism—it has usually been considered necessary to ensure that the strain used shall be highly virulent. On the other hand, M. Nicolle (1908[2]) found that, so far as could be ascertained, the amount of endotoxin varied little with the virulence from one strain to another. The term ‘ virulence ’ is considered by this author to comprise two factors, viz. parasitic adaptation and toxicity. The different effects on the animal produced by different strains thus depend primarily on their power of adaptation to the host, though fundamentally the effect is due to the toxicity factor.

Microscopic Changes in the Tissues Resulting from the Action of B. mallei *and its Products.*

Among the earlier authors who investigated experimentally the histogenesis of the glanders nodule were Baumgarten (1890) and Tedeschi (1893). The former described a proliferative type of lesion bearing a strong resemblance to certain tuberculous lesions. Tedeschi on the other hand pointed out that in glanders the primary change is a degenerative one, marked by an early focal necrosis.

The nature of the reaction of the lung tissue of horses to *B. mallei* has been studied by Leclainche and Montané (1893), by Schütz (1894) and by M'Fadyean (1895) among others. At points where the bacilli are arrested and have begun to multiply, areas of broncho-pneumonia develop, the cell reaction of which is essentially polynuclear, in contradiction to tuberculosis, where the reaction is chiefly mononuclear. Under the influence of the bacterial products the reacting cells disintegrate, but some time elapses before the fragmented nuclei lose their affinity for chromatin stains. Consequently in sections of recent glanders nodules stained with hæmatein and eosin, the central portion appears as an area deeply stained with hæmatein. The degeneration occurring in glanders nodules was termed ' chromatotexis ' by Unna, though the change does not differ essentially from that seen in other affections or more commonly known as ' karyorrhexis '. The lung tissue surrounding this central zone reacts with acute croupous or catarrhal changes of varying degrees of intensity. As the nodule grows older, the necrotic material gradually loses its affinity for nuclear stains and become enclosed in a capsule of connective tissue. The microscopic changes occurring in other organs are similar in type to those found in the lung.

Duval and White (1907) found with rabbits and guinea-pigs that the microscopic changes in glanders are dependent to some extent upon the virulence of the infecting organism. Such differences in virulence are no doubt responsible for the different histological appearances described by the earlier experimenters, Baumgarten and Tedeschi. Highly virulent bacilli produce cell necrosis and disintegration, which is followed by an invasion of polynuclear cells ; attenuated bacilli produce an early tissue proliferation with the formation of epithelioid and giant cells. Between these two extremes every grade of lesion may be met with.

Factors concerned in the Mechanism of Spread of Infection.

Viability of the Organism.

B. mallei possesses in general low resistance to adverse agencies such as light, heat and desiccation, and consequently its viability in the outer world is of a comparatively low order. Many of the observations on this subject have not been made under precise experimental conditions, and consequently the results obtained by different authors have been somewhat variable. So far as desiccation is concerned, most of the attempts of the earlier workers at setting up the disease by blowing into the nostrils of horses and asses infective nasal discharge which had been dried were usually fruitless. Löffler (1886) carried out experiments with silk threads, which were allowed to dry after they had been soaked in pus from glandered guinea-pigs or in spleen material from field mice. After a few days exposure, the bacilli on the threads were incapable of development. Cadéac and Malet (1886) also found that pus or nasal discharge dried at room temperature was usually non-infective after 3 days. It was observed further that the duration of infectivity of dried material kept in the open air was dependent to some extent upon the weather. In warm, dry weather the virulence disappeared 3 to 9 days more quickly than when the weather was cold and moist. The effect of the rate of drying was also investigated by these authors. Pus which was dried quickly maintained its virulence longer than when the material was dried slowly. In one series of experiments, nasal discharge dried rapidly (31° C. 2 hours) was infective after 6 days, while a portion of the same discharge left free in the open air was inactive after 3 days. Izkowitsch (1888) corroborated the effect of 'weather' by finding that, while discharge spread out in a thin layer in a room lost its infectivity after 3 days, the same discharge kept in a moist stall was infective after 6 to 11 days.

In order to investigate the effect of desiccation upon bacilli from culture, Löffler (1886) soaked silk threads in a broth emulsion of growth from solid media (serum or potato), and then allowed them to dry quickly on glass. After 4 days bacilli on the threads were still alive, after 14 days the results were uncertain, and after 3 weeks the organisms usually failed to develop. In one experiment, however, threads which had been quickly dried were shown to be infective for field mice after 89 days.

On the surface Löffler's experiments suggest that *B. mallei* in culture is more resistant to desiccation than when contained in discharges, and the work of subsequent observers served to confirm the fact. For example, in a series of parallel experiments with cultures and with nasal discharge Nowikoff (1895) found that the organisms died 6 to 12 days earlier in the discharge than in the culture. No account, however, appears to have been taken of the relative numbers of organisms submitted to desiccation. In other experiments Nowikoff observed that the duration of survival depended upon the conditions of light under which the dried material was kept. If exposed to light the organisms died 4 to 14 days more quickly than if kept in the dark.

The virus is very resistant to cold, e.g. it survives exposure to liquid air (−185° C.) for 80 minutes (Wladimiroff, 1913, p. 1093). On the other hand, it is quickly destroyed by heat, e.g. at 55° C. for 10 minutes.

Precise information regarding the survival of *B. mallei* in water would be of great practical value, owing to the frequency with which the disease has been spread among equines by water supplies, e.g. by horse troughs. According to Cadéac and Malet (1886), the organism in pus may remain alive in clean water for 18 days at ordinary temperatures, but the possibility remains that under more favourable conditions survival of the organism may be more prolonged.

Distribution of the Organism.

Distribution in the outer world. Owing to its obligatorily parasitic nature the distribution of the organism is largely restricted to the particular localities in which the disease exists. Since in the case of equines the bacilli are excreted in greatest numbers from lesions of the skin and nasal cavity, objects liable to be contaminated with such excretions are to be looked upon as especially dangerous. Such objects are the food and feeding mangers, watering troughs and buckets, bedding, harness and brushes or other grooming utensils.

The possibility of the organism being excreted with the urine has been referred to, though its survival in this medium is not believed to extend beyond 48 hours. In order to determine whether development would occur in the fluids found in horse stalls, Löffler (1886) instituted a series of experiments in which decoctions or watery extracts of hay, straw or horse manure after neutralization were sown with *B. mallei.* No growth was observed when these media were incubated at 37° C.

Distribution within the body. The organism is usually confined to the lesions and their discharges, and it is only in very acute cases that bacilli are sparingly present in the blood. As has been mentioned before, glanders is essentially a disease in which infection is liable to remain latent within the body. Lesions frequently exist for months in the internal organs, especially in the lungs, without important disturbance of health. Animals with such lesions must be held to be, at any rate potential, disseminators of infection.

Variation in Virulence of the Organism.

Reference has been made to the possibility of enhancing the virulence of the organism experimentally by means of animal passage. It is possible therefore that a similar rise in virulence will occur during natural passage, and that this factor in itself may be chiefly responsible for a more than usually rapid or extensive spread of the disease. This hypothesis finds support in observations to the effect that strains of varying virulence may be isolated from cases of the natural disease. Actually of course the issue is complicated by other factors, such as variable susceptibility.

Increased Susceptibility.

While different species of animals undoubtedly vary in their natural susceptibility, there is also a variation within a given species due to different factors. Susceptibility varies in some cases with age, thus young cats and dogs are distinctly less resistant than adults. Factors such as fatigue or other diseases are also believed to increase the susceptibility of horses.

Diagnosis.

DIAGNOSIS DURING LIFE.

Microscopic detection of B. mallei.

B. mallei possesses no distinctive characters by means of which it may be recognized when mixed with other bacteria. For this reason the possibilities of making a diagnosis by microscopic methods alone are limited. There are instances, however, particularly in equines, in which a reasonably certain diagnosis may be made in this way. These are cases in which smears of pus from previously unopened skin lesions show small numbers of Gram-negative bacilli of the proper size and characters, unmixed with other species of bacteria.

Cultural detection of B. mallei.

This method is also of outstanding value only in cases where unopened lesions are present on the skin. Pus from such suspected lesions should be plentifully sown on solid serum and on potato slopes. The first point which raises a suspicion that the case may be one of glanders is the late appearance of naked-eye growth in primary culture. Growth is not likely to be seen until the second day, and frequently three days elapse before it becomes visible in the form of isolated colonies of characteristic appearance. Importance is to be attached to the colour and texture of the growth and to its non-spreading nature. Isolation may also be attempted from material from open sores, but difficulties are to be expected owing to the slow rate of growth of *B. mallei* in primary culture.

In some very acute cases in man diagnosis has been based on cultures from the blood.

Animal Inoculation.

At the present time inoculation tests are carried out on guinea-pigs. If pus from unopened lesions is available, this may be inoculated intraperitoneally, otherwise it is advisable to inoculate subcutaneously as well. In either case several good-sized guinea-pigs should be used, as the organisms may be scanty in number, of low virulence, or mixed with other bacteria, with the result that some animals may escape infection.

Glanders in the guinea-pig. The Straus reaction, which appears in male guinea-pigs after intraperitoneal inoculation of virulent material, consists of a tense enlargement of the scrotal sacs, due to a suppurative infection of the tunica vaginalis. In typical cases, after intraperitoneal inoculation of small amounts of glanders pus or culture, the enlargement becomes apparent within 24 to 48 hours, and by the third to fifth day has exceeded the size of a walnut. The testes are then found to be cemented in the scrotum and cannot be pressed back into the abdominal cavity. The animal becomes emaciated and death ensues 2 weeks or so later, though sometimes as early as the fourth to eighth day. There are considerable variations from this course depending upon the number and virulence of the infecting organisms. Thus the reaction may not appear for 12 days; in other cases where the organisms have lost greatly in virulence, the scrotal lesions may burst and discharge pus and the animals may survive. In still other cases the ectopic form may supervene. On the other hand, if the bacilli are of high virulence, exceedingly small doses intraperitoneally are capable of infecting. M. Nicolle (1906[1]) succeeded in producing infection with a quantity of moist culture as small as one-millionth of a centigram. Owing to the tendency of the glanders bacillus to localize on the serous coverings of the testis, it has been suggested that a more rapid diagnosis may be made by killing the animal 5 or 6 days after intraperitoneal inoculation and making cultures from the tunica vaginalis. In such cases, even though other organisms are present in this situation, glanders bacilli are said to predominate.

On post-mortem examination of a guinea-pig showing a Straus reaction, the omentum and general lining of the peritoneal cavity are as a rule free from visible alterations, though there may be a certain amount of exudate present. Lesions are confined to the serous coverings of the testes, the layers of which are separated by yellowish and somewhat inspissated pus. At early stages of the disease the testicular substance is normal in appearance, but if death is delayed the tissue may be partially destroyed by suppuration. Lesions also occur in other parts of the body, most constantly in the spleen and lungs, and in advanced cases in the liver and lymphatic glands. These take the form of greyish nodules, which in their general macroscopic and microscopic characteristics resemble those described in horses. More rarely there are observed joint suppurations and ulceration of the nasal mucous membrane.

It is always advisable to confirm the presence of *B. mallei* in the exudate around the testis by microscopic and cultural examination, because, contrary to the original belief of Straus, several different species of bacteria are capable of producing scrotal changes in male guinea-pigs. Of these the more important are the bacillus of Preisz-Nocard, *B. pyocyaneus*, *Sporotrichum sp.*, and *B. whitmori*. Panisset (1910) noted that a Straus reaction may also be produced at times by the intraperitoneal injection of tubercle or diphtheria bacilli. Provided that the reaction is controlled microscopically and culturally, however, the facts just stated do not seriously diminish the value of the test.

In typical cases subcutaneous inoculation with glanders material produces local abscess formation with enlargement of the neighbouring lymphatic glands, and is followed by generalization of the disease with lesions in the spleen and less constantly in the lungs and liver. Death occurs 2 to 6 weeks later, with or without a Straus reaction. In cases where the material is highly contaminated, it has been suggested that guinea-pigs should be inoculated subcutaneously in the first place. As soon as enlargement of the local lymphatic glands is well established, these may be extirpated and used for the intraperitoneal inoculation of other guinea-pigs. Alternatively, the material may be introduced by scarification, but owing to the uncertainty of this method at least four animals should be used.

Though the bacteriological methods which may be used for the diagnosis of glanders during life have been described separately, all three methods would be used in practice. In the case of equines, great assistance in diagnosis can also be obtained from clinical signs, viz. nasal discharge accompanied by induration of the submaxillary lymphatic glands, farcy, and lymphangitis affecting a limb. In man clinical examination in conjunction with the history of the case may also provide useful information.

Tests for Allergy.

The use of mallein as a diagnostic agent has been widely practised and may be looked upon as the simplest method by which the existence of latent disease in equines may be ascertained. The reagent has been used by subcutaneous and cutaneous inoculation and by instillation into the conjunctival sac.

The subcutaneous mallein test.

This was the first method used and is still regarded by many as the most reliable in practice. The dose of diluted mallein first recommended was 2·5 c.cm., containing 0·25 c.cm. crude mallein, the injection being made subcutaneously in the neck. It has been repeatedly proved, however, that 1·0 c.cm. diluted mallein, containing 0·1 c.cm. crude mallein, is sufficient to produce pronounced reactions in glandered horses. The dose of alcohol-precipitated mallein recommended was 0·06 to 0·1 gm., dissolved in sterile distilled water immediately before use. A severe positive reaction is denoted by (*a*) signs of general body disturbance, such as increased pulse and respiration, shivering and extreme depression, (*b*) a rise of temperature, and (*c*) the formation of a swelling at the site of injection. The temperature of the animal should be taken and the site of the injection inspected at the ninth, twelfth and fifteenth hours, and in cases where the reaction is doubtful, it is advisable to make further observations at the twenty-fourth and forty-eighth hours or even later. In less severe reactions general effects may be absent and there may be very little rise of temperature. The greatest importance is attached to the presence of a local swelling, since in mild reactions this may be present alone. According to M'Fadyean (1893), in deciding whether a local swelling should be looked upon as diagnostic, attention should be paid to the following points. If the case is one of glanders, the local swelling 24 to 48 hours after the mallein is injected should measure at least 5 inches (13 cm.) in its shortest diameter, should show fairly well-defined edges, and should be tense and painful on palpation. The lymphatic vessels leading to the neighbouring lymphatic glands may be visibly enlarged. After the forty-eighth hour, the size of the local swelling gradually declines. In healthy animals the swelling reaches a maximum size of 3 inches (8 cm.) or less within the first 12 hours, and then rapidly declines so that by the twenty-fourth hour it has nearly or quite disappeared. In cases where the local swelling does not possess all the characteristics just described, and in which there is no decided rise of temperature, the diagnosis cannot be regarded as established. The first decided rise of temperature usually takes place about 6 to 8 hours after injection, the maximum being reached 4 to 6 hours later, after which there is a gradual fall to normal. Exceptionally, the temperature may not rise for about 20 hours after injection. It was noted by Tatray that in 395 positive cases, the highest temperature was recorded at the sixth hour in 2, at the ninth hour in 74, at the twelfth hour in 174, at the fifteenth hour in 114, at the eighteenth hour in 23, and at the twenty-first hour in 8. In the experience of M'Fadyean, a horse may be considered glandered if the temperature rises from 101° F. (38° C.) or less to 103° F. (39·5° C.) or more at the tenth to fourteenth hour after injection, and if there is a painful local swelling not less than 4 inches (10 cm.) in diameter at the twenty-fourth hour. Nine times out of ten, one would be correct in making a positive diagnosis if in such cases the temperature or the local reaction were considered separately. If the local swelling measures 3 to 4 inches (8 to 10 cm.) and there is a rise of 2° F. (1·1° C.), the animal may be said to be probably glandered. In 80 per cent. of glandered horses the temperature rises to 104° F. (40° C.) or higher, and the local swelling reaches 4 to 5 inches (10 to 13 cm.) in diameter at the forty-eighth hour. On the other hand, in 80 per cent. of healthy horses the temperature does not rise above 101·5° F. (38·6° C.), and in all the local reaction has practically disappeared by the second day. In cases where the initial temperature is high, e.g. 103 to 104° F. (39·5 to 40° C.), little reliance can be placed upon temperature observations.

The use of mallein for diagnosis of the disease in man has been referred to by several authors (V. Babes, 1894; Bonome, 1894). Bonome noted in one instance that the subcutaneous administration of three drops of mallein was followed five hours later by severe chill, with a temperature rise at the twelfth hour to 103·5° F. (39·8° C.), accompanied by great systemic disturbance.

The ophthalmic mallein test.

In this method 0·2 c.cm. mallein which has been concentrated to one-tenth of its volume and which contains no preservative is instilled into the conjunctival sac. Precipitated mallein dissolved in distilled water to 5·0 per cent. concentration has also been used. Of this solution two drops represents a suitable dose, though positive reactions have been obtained with amounts of dry mallein as small as 0·0005 gm. Observations should be made upon the tested eye at the ninth, twenty-fourth and forty-eighth hours, and comparison made with the untested or control eye. In healthy animals practically no change occurs. When the reaction is positive, the conjunctiva is seen to be swollen and congested, and the lachrymal secretion greatly increased. The result, however, should not be regarded as distinctly positive, unless a considerable amount of mucopurulent discharge is present. The changes which are regarded as diagnostic begin to appear 4 to 6 hours after the mallein is instilled and are still present 24 hours later. Reacting animals may also show a slight rise of temperature and this may be a confirmatory sign in doubtful cases.

Cutaneous mallein tests.

The *cuti* or *dermo reaction* as applied to glanders was first introduced by Vallée (1907[1]). To perform this test an area of skin at the side of the neck is shaved, scarified in three places and concentrated mallein rubbed into two of them, the third place serving as a control. A positive reaction is indicated by a painful and somewhat persistent œdematous thickening of the lines of scarification. The changes begin to appear at about the sixth hour, increase during the following 24 hours and then gradually disappear. The degree of the reaction is judged by the thickness and persistence of the swelling in comparison with the control area.

The intradermo reaction. In this test an area of skin at the side of the neck is shaved and intradermal injections of 0·1 c.cm. concentrated mallein are made at several points. In a glandered animal, firm, painful thickenings persisting for 24 to 48 hours may be expected to appear at the sites of injection.

The intrapalpebral mallein test.

This test was devised in 1914 by Lanfranchi with the object of combining the subcutaneous and ophthalmic methods in one operation. 0·25 c.cm. crude mallein diluted at the time of use with saline to 2·5 c.cm. was injected subcutaneously into the lower eyelid. In healthy horses this was followed by a somewhat transitory local œdema, with insignificant changes in the conjunctiva. In glandered animals, on the other hand, there was usually a strong reaction, consisting of a firm, painful swelling of the injected eyelid, the swelling frequently involving the whole orbital area. In addition, the changes described as characteristic of a positive ophthalmic reaction were also observed, viz. conjunctivitis with muco-purulent exudation. In typical cases the reaction persisted in an acute form for 3 or 4 days and was accompanied by a rise of temperature.

The intradermo-palpebral mallein test.

This method of using mallein was introduced in 1914 by Drouin and Naudinat, and was suggested by the use of a similar test for tuberculosis. The method differs from that of Lanfranchi in that the injection is made intradermally close to the edge of the lower eyelid and in that a smaller dose of mallein is used. Drouin recommended the injection of 0·1 c.cm. of a saline dilution of crude mallein (crude mallein 1 part and saline 3 parts), the saline being added at the time of use.

In healthy animals there is either a slight œdema or no appreciable alteration in the injected eyelid. In a typical positive reaction the local changes are similar to those following the intrapalpebral test.

The Practical Diagnosis of Glanders by the Use of Mallein.

Specificity of mallein. Mallein may be regarded as specific, inasmuch as healthy animals can tolerate considerable doses without appreciable effect, while those affected with glanders show pronounced reactions to small amounts of the reagent. This broad statement while usually true requires some qualification. Thus glandered animals at the time of being tested may fail to respond to any method, while healthy equines, or equines affected with other diseases than glanders, e.g. strangles or purpura, may show atypical or false reactions. Such atypical reactions are denoted by œdematous swellings at the seat of injection, which are not particularly tense or painful and are usually transitory, disappearing within 48 hours. Sudmersen and Glenny (1908) noted that horses in the course of being immunized with diphtheria toxin or other bacterial products were liable to display such atypical local reactions, but without any accompanying rise of temperature. There are also occasions on which horses affected with glanders fail to react. In the first place, there is the interval immediately after infection during which horses are incapable of reacting. In the case of the subcutaneous test this interval does not greatly exceed a week ; with the ophthalmic method the evidence suggests that, though variable, it may be rather longer, but probably not more than 19 days. Secondly, an atypical reaction or no reaction at all may occur in horses with widespread lesions of farcy, or in horses and other equines, particularly mules, in which the disease process is very acute.

Reliability of the various methods of using mallein. Apart from the somewhat exceptional cases just mentioned in which glandered animals do not react, mallein is a most useful diagnostic agent. The reliability depends to some extent, however, upon the particular method of testing. The *cutaneous methods* have not been applied extensively, owing to their general unreliability. Vallée (1907[2]) found that the fine skin of some healthy horses showed reactions which could not be distinguished with certainty from the specific reactions of glandered horses. Frankly positive results can alone be accepted as indicating infection, and in many cases the results would have to be regarded as doubtful. Authors differ as to the amount of confidence which may be placed in the *ophthalmic method.* The reason for the difference of opinion is probably due in part to the different forms of mallein used, and in part to the difficulty of reading the results in doubtful cases. Schnürer (1911) in a series of 203 glandered animals noted positive reactions in 186, that is, the result corresponded with the post-mortem findings in 91 per cent. of cases. In a series of 321 glandered horses recorded by Lührs (1927, p. 65), 97 per cent. had reacted positively and the remainder were doubtful or negative. In the United States the ophthalmic method appears to have given complete satisfaction. K. F. Meyer (1913) regarded the test as easy of application and reliable, though atypical reactions occurred occasionally, such as the sudden appearance and disappearance of the eye changes, or changes noticeable for the first time after 24 hours. No great constancy was observed in the duration of the reaction, which might be any time between six hours and three days.

The use of the ophthalmic test dispenses with the necessity of making repeated temperature observations. This important consideration increases the value of the test in cases where large numbers of animals are concerned, e.g. army horses under field conditions. A certain proportion of doubtful reactions are to be expected at the first test, and animals showing these may be retested in the same eye after an interval of 24 hours. In fact, in glandered animals the first instillation of mallein sensitizes the conjunctiva, so that the second reaction becomes more pronounced. This hypersensitive condition of the conjunctiva does not develop in healthy horses as the result of repeated tests. According to Meyer (1913), the reaction is liable to become less distinct after 3 or 4 tests, so that more than three retests should not be applied to the same eye.

The result of an ophthalmic test is not influenced by a previous subcutaneous test. On the other hand, there is every advantage in controlling doubtful eye reactions by the subcutaneous method, as repeated testing of the eye does not interfere with the capacity of the animal to react to mallein subcutaneously. Moreover, there appears to be a tendency for subcutaneous injections of mallein to revive inflammatory changes in an eye previously tested. There is certainly some evidence (Lührs, 1927, p. 104) that horses may continue to react for long periods to the ophthalmic method, and even after they have ceased to react to the subcutaneous method or after serum antibodies have disappeared. In this connection it may be observed that in certain instances M'Fadyean (1901) recovered living glanders bacilli from the lung lesions of horses which had ceased to react to subcutaneous mallein. Marxer (1919) concluded that a positive result to the ophthalmic test was absolute evidence of contact of the tissues with living bacilli, since this test always gave a negative result in animals injected with dead bacilli.

The *intradermo-palpebral method*, like the ophthalmic, is suitable for the testing of horses *en masse*. During the recent war the method was applied extensively for the control of glanders in the French and British Armies. Fayet (1915), who reported on the use of the test in 15,000 horses, stated that failures were uncommon, but that reactions are occasionally delayed beyond the thirty-sixth hour.

So far as general reliability is concerned, a wide experience has shown the high value of the *subcutaneous test*. Infected horses are capable of reacting several times in succession to subcutaneous injections of mallein, and tolerance to this reagent is less readily acquired than to tuberculin. Leclainche (1892), however, found that on repeatedly testing with mallein, the intensity and especially the duration of the reactions regularly decreased. While therefore a second subcutaneous test can be applied within a week of the first, it is a wise precaution where possible to allow three or four weeks to elapse.

Serological Tests for Glanders.

The principal serological methods which have been used are those of complement fixation, agglutination and precipitation.

The complement-fixation test.

Since the discovery by Schütz and Schubert (1909) that the blood-serum of glandered horses possesses complement-fixing properties, the test has been studied by many from the point of view of diagnosis. The majority of observers have followed the original recommendation of Schütz and Schubert in using for the routine test falling amounts of serum from 0·2 to 0·01 c.cm. in the presence of suitable amounts of antigen, complement, &c. A diagnosis of glanders is warranted if the serum gives fixation, even though incomplete, in quantities of 0·1 to 0·2 c.cm. A small proportion of samples of horse-serum, and a larger proportion of samples of ass- and mule-serum, are found to be anticomplementary after inactivation at 56° C. To inactivate such sera, the temperature should be raised to 62 to 63° C. for half an hour. As a general rule, heating above this temperature should be avoided, owing to the possibility of injuring the specific reacting substances, particularly in sera which are weakly positive. Sera which remain anticomplementary after being heated to 63° C. should be tested by the conglutination method. The antigen recommended by Schütz and Schubert was prepared as follows. Young cultures on glycerin agar contained in Kolle flasks were washed off with saline (30 to 50 c.cm. per flask), the emulsion heated at 60° C. for 3 hours, shaken for 4 days, and a clear extract obtained by centrifuging. If stored in a dark, cool place, the extract keeps its antigenic properties for 4 to 6 weeks, but it was considered to be an advantage to store the extract about 8 days before using it for the first time. Mohler and Eichhorn (1911), by following the lines of Schütz and Schubert, obtained an antigen which could be used for 2 or 3 months. The antigen should be of such strength that 1·0 c.cm. of 1 in 100 dilution represents approximately one-half the anticomplementary dose in the presence of one m.h.d. complement, two m.h.d. hemolytic amboceptor, and 1·0 c.cm. 5·0 per cent. sheep blood corpuscles.

Wade (1913) reported that antigens prepared on these lines were liable at times to be unstable and were not efficient for detecting some clinical cases of glanders. This author used polyvalent antigens prepared in the following manner. Eleven strains of *B. mallei* were grown on slightly acid glycerin agar, the growths emulsified in distilled water, heated at 56° C. for half an hour, passed through a Berkefeld filter, and the filtrate heated at 56° C. for 2 hours on each of three successive days. Before use, the antigen was made isotonic with 9·0 per cent. sodium chloride solution. Pfeiler (1915) also emphasized the necessity of using for the complement-fixation test either polyvalent antigens or monovalent antigens known to be of high value.

Liquid mallein, Foth's precipitated mallein, as well as alcoholic or antiformin extracts of bacilli, have been found to be less suitable as antigens.

Practically all observers who have worked with the complement-fixation test in equine glanders are agreed that the reaction is of great value for diagnosis, though two tests at an interval of a fortnight may be required in some chronic cases, owing to the periodic rise and fall in serum titre.

The serum from cases of human infection has also been shown to possess complement-fixing properties, sometimes in amounts as low as 0·02 c.cm.

The agglutination test.

A critical review of this and other serum reactions in glanders has been contributed by Pfeiler (1910). Schütz and Miessner (1905) were the first to apply it on a large scale for the diagnosis of the disease in horses.

The test may be carried out by either the usual naked-eye or microscopic methods, though the former has been preferred by the majority of observers. For the sake of safety emulsions may be heated to 60° C. for 2 hours without any noticeable alteration in agglutinability. The final dilutions of horse-serum in the emulsion should be somewhat as follows: 1 in 100, 200, 400, 500, 600, 800, 1,000 and 2,000, and the usual serum controls should be included. The tubes are incubated at 37° C. for 24 to 36 hours prior to the reading of results.

The interpretation of the results is made more difficult by the existence in normal horse-serum of non-specific agglutinins in relatively high titre, though in the same serum dilutions normal agglutinins produce less rapid clumping than specific agglutinins. The suggestion of Oyuela (1912) that normal agglutinins, unlike specific agglutinins, can be inactivated by heat (56° C. half an hour) is disputed by Hutyra (Hutyra and Marek, 1926, p. 859), who found that the effect of heat was irregular. Strains of *B. mallei* are known to vary somewhat in their agglutinability from time to time (M'Fadyean, Schütz and Miessner). Usually they become less agglutinable on subcultivation, and three-weekly passage through susceptible animals (guinea-pigs) has been recommended as a means of preserving agglutinability (Schütz and Miessner). Wade (1913) advised that the emulsion should be prepared from several strains. After storage on ice for 8 to 10 days, such an emulsion could be used for periods up to 2 or 3 months. The reaction may be hastened by centrifuging the mixtures of serum and emulsion after incubation at 37° C. for half an hour to 2 hours. Povitzky (1918) points out, however, that there is no real necessity to resort to centrifuging, provided that a highly agglutinable strain of the organism is selected. If such a strain is used, the result may be read after 2 hours incubation in a water-bath at 37 to 42° C. According to M'Fadyean, the critical tube in the series by the macroscopic method is that containing the serum in final dilution of 1 in 500. Most normal horse-sera will not agglutinate in dilutions weaker than 1 in 300, while the serum of most glandered horses will agglutinate in dilutions weaker than 1 in 500, and will frequently agglutinate at 1 in 1,000 or 1 in 2,000. From statistics furnished by Schütz and Miessner (1905), involving agglutination tests of 1,911 healthy horses and 298 glandered horses, it is evident that a titre of 1 in 1,000 may be regarded as practically diagnostic of the disease, while titres between 1 in 500 and 1 in 800 can only be regarded as suspicious. In natural cases of glanders in the horse there is usually considerable fluctuation in the agglutinating power of the serum from time to time, the fluctuations probably depending upon periodic exacerbations in the disease process. For this reason a single agglutination test cannot be relied upon for accurate diagnosis in equines when the disease has passed the acute stage. If it were necessary to rely solely upon the agglutination test, two or three tests at intervals of three weeks would be required. Importance would then be attached not only to the actual titres reached but also to their fluctuating character. In cases in which the disease has assumed clinical characters, the agglutination titre remains more constant and at a high level.

In human glanders agglutinins have been observed to reach a titre of 1 in 1,000 to 1 in 2,000 in acute cases, while normal agglutinins are present to a titre of 1 in 100 (Collins, 1908). The agglutination titre falls very low when the disease passes into the chronic stage.

The precipitin test.

As a means of diagnosis this method has no special value. The precipitin titre is highest shortly after infection and falls very low in the chronic stage.

In carrying out the test, it is advised that the serum should be diluted from 1 in 2 to about 1 in 40, and slowly added to the tubes containing the antigen. The results may be read after the tubes have stood at room temperature for an hour, or the mixtures may be incubated at 37° C. for 30 to 40 minutes. The following antigens have been used. Growths from solid media extracted in saline, carbolized saline or carbolized horse-serum ; dilutions (1 in 10) of crude mallein ; 10·0 per cent. solutions of Foth's dry mallein. Konew (1910) strongly recommended a product described under the name of ' mallease '. This was prepared by dissolving growths from solid media in 8·0 per cent. antiformin and subsequently neutralizing and filtering. This product has also been favourably reported on by Zurkan (1911).

The conglutination test.

This reaction depends upon the existence in the normal serum of certain species of animals of a hypothetical substance known as ' conglutinin ', which is capable of causing agglutination of red corpuscles in the presence of free complement. A negative result is indicated by agglutination (conglutination) of the red cells and a positive result by absence of agglutination.

The reaction was first applied to the diagnosis of glanders by Pfeiler and Weber (1912), and their results have been confirmed in the main by several others. Pfeiler and Weber noted that with six glandered horses, conglutination was inhibited by the serum in amounts as low as 0·05 to 0·03 c.cm. Schoening (1917) found that in 134 positive cases and in 174 negative cases in horses, the results were correct and agreed with those of complement fixation. Pfeiler and Weber believed that in certain cases the conglutination test might be even more reliable than the complement test for diagnosis, since, though conglutination developed at a later stage, it was more persistent than complement fixation. Waldmann (1914, 1916), however, could not agree that the conglutination test was of special value for the detection of chronic cases, while according to Lührs (1927, p. 93) the serum of about 10 per cent. of healthy horses or of horses suffering from other diseases than glanders reacts positively. On the other hand, it is usually agreed that the reaction in question is especially applicable to the ass and mule, the serum of which is liable to be more anti-complementary than that of horses. For the purpose of this test it is unnecessary to inactivate the serum of the ass or mule at a higher temperature than 58° C. if it is heated for half an hour.

Influence of Mallein Injections upon the Titre of Serum Antibodies.

• There is general agreement that injections of mallein stimulate the production of specific complement fixing, agglutinating and precipitating properties in the blood. Within a week of injecting the mallein the titre begins to rise both in healthy and in glandered animals, but in the case of the latter the rise is usually higher than in healthy animals or in animals in which the initial titre was low. Reinhardt (1913) states that the agglutination titre is influenced more easily and for a longer period than that of other antibodies, and that precipitins are the least affected. Costa and Fayet (1911) found that, as early as 24 hours after injection of mallein, precipitins could be detected in the undiluted serum. They expressed the opinion that some of the favourable results in diagnosis recorded with the precipitin test may have been due to disregard of the influence of mallein injections. Ciuca (1910) found that the serum of rabbits possessed complement-fixing properties 3 days after a subcutaneous injection of 100·0 mgm. of precipitated mallein, fixation occurring with precipitated mallein as antigen in a dilution of 1 in 5,000. Brocq-Rousseu, Forgeot and Urbain (1923) detected similar properties in rabbit-serum even after the mallein was given by the mouth. In the case of horses, the same authors (1921) found that a first subcutaneous injection of 2·5 c.cm. diluted mallein produced complement-fixing properties in the serum of 4 out of 5 animals. The specific properties appeared within 4 to 8 days after injection and had disappeared by the fortieth day. The titre reached was related in some measure to the amount of mallein injected. According to Lührs (1927), repeated ophthalmic tests in equines, as well as the administration of large amounts of mallein *per os* or *per rectum,* do not induce any specific change in the serum. Fröhner and Habersang (1920) mention that intradermal injections of mallein produce specific alteration in the serum of horses, and this fact has been confirmed by others.

The Diagnosis of Equine Glanders in Practice.

The diagnosis of latent glanders in equines during life may be made either by the use of mallein or by means of serum tests. From what has been said in the last section it is clear that the use of mallein, other than by the ophthalmic method, is to be avoided in effectives in which serum tests are contemplated.

When comparatively small numbers of animals are to be tested, mallein may be applied subcutaneously with great advantage. Experience in Great Britain and elsewhere has shown that by this method alone the disease may be eradicated. In order to save labour on occasions when large numbers of animals have to be tested under field conditions, it has been considered more convenient to use either the ophthalmic or the intradermo-palpebral method. The interpretation of the results is sometimes rather difficult, and a certain percentage of intermediate or doubtful reactions is to be expected. In such cases a second test of the same eye may be made 24 hours later, or the results may be controlled by the use of mallein subcutaneously.

In certain parts of the world, and particularly in Central Europe, serum tests (complement fixation and agglutination) have been widely used instead of mallein. For the ass and mule, the sera of which are liable to be highly anticomplementary, the conglutination test is recommended. In the case of horses chief reliance has been placed on the complement-fixation test, though owing to the fluctuations in titre which occur in chronic glanders a single complement-fixation test cannot always be relied upon for diagnosis. The disease, however, may be controlled by the complement-fixation method alone if two or three tests are carried out at fortnightly intervals. In the past it has been usual to employ the complement fixation and the naked-eye agglutination tests in conjunction, though it is only in some very recent infections that the agglutination test may be expected to provide additional information. Other observers, e.g. Lührs (1927, p. 103) prefer to test animals with ophthalmic mallein and at the same time to apply the complement fixation test. Detailed information as to the comparative diagnostic value of ophthalmic mallein, complement fixation and agglutination has been compiled by Miessner (1912).

Diagnosis after Death.

This will rest upon naked-eye and histological examination of the lesions and upon bacteriological methods.

In typical cases in equines, it is often possible to make a reasonably certain diagnosis by naked-eye examination of the lesions. The greatest importance is attached to the appearance of pulmonary lesions, and the existence of lesions in other organs, such as the spleen and submaxillary glands, or of nasal ulceration and farcy, will assist in confirming the diagnosis. Efforts should also be made to confirm the diagnosis by bacteriological methods, which include microscopic and cultural examinations and the inoculation of animals. Bacteriological methods, however, may fail owing to the scarcity of the causal organisms. For this reason in part, histological studies using tissue stains are often of great value, special importance being attached to the occurrence of chromatotexis.

Differential Diagnosis.

In Equines.

Glanders in equines is to be distinguished from certain other conditions which affect the skin, the most important of which are the following :

Chronic suppurative infections of the skin and subcutaneous tissues are not of infrequent occurrence in horses. Such infections may be associated with the presence of streptococci, staphylococci (*Botriomyces*) or of the diphtheroid bacilli of Preisz-Nocard.

Epizootic lymphangitis. This is a chronic, contagious disease of equines, the skin lesions of which closely resemble those of farcy. The abscesses contain creamy pus, in which may be detected microscopically *Cryptococcus farciminosus* Rivolta, an organism belonging to the genus *Blastomyces.*

Sporotrichosis is another chronic skin affection, due to *Sporotrichum sp.*, the conidia of which may be detected in the pus on microscopic examination.

Bacteriological examination of material from the lesions, combined with failure of the animals to respond to mallein, serves to differentiate these conditions from glanders.

Of conditions affecting the respiratory tract which bear some similarity to glanders may be mentioned the following:

Nasal catarrh of a more or less chronic nature is of common occurrence in horses and is usually accompanied by swelling of the submaxillary lymphatic glands.

Strangles resembles glanders, inasmuch as in the acute form there is a nasal discharge with abscess formation in the submaxillary glands. Unlike the submaxillary enlargement in glanders the abscess frequently bursts spontaneously, and the streptococci which are associated with the condition may be detected in the pus.

In equines after death lesions are frequently found in the lung tissue which bear some resemblance to glanders nodules and which arise as the result of irritation by *worm parasites.* In point of size these formations are usually smaller than peas, and on section the centres are found to be calcified. Microscopically, the reaction on the part of the lung tissue is seen to be of a chronic nature, the outer part of the nodule being composed of fibrous tissue. In the earlier stages remains of the worm parasites may be seen and frequently large numbers of eosinophile cells are present. The two important differential characteristics of worm nodules are the presence of eosinophile cells and the extensive calcification.

It is possible that the *pulmonary lesions of tuberculosis* may be mistaken for those of glanders. Such lesions, however, are usually miliary in distribution; apart from this the diagnosis may be established by bacteriological and histological examination.

Differential Diagnosis in Man.

Glanders in the human subject has to be distinguished from *necrotic and pyæmic conditions* of a more or less chronic character, due to other organisms and affecting tissues such as skin and bone. In the past the disease has been mistaken for *syphilis* and *tuberculosis.* The onset of glanders in man is frequently marked by very acute symptoms of obscure origin. The differential diagnosis from such diseases as *typhoid* and *paratyphoid fever, acute rheumatism* and *acute osteomyelitis* then comes into question. Certain cases in the earlier stages are said to have borne a distinct resemblance to the papular stage of *variola.* In 1913 Whitmore in Malay described under the name of '*melioidosis*' a disease of man which simulated glanders. The causal organism, *B. whitmori*, has been shown to belong to the *mallei* group, but can be readily distinguished by its motility and by the wrinkled nature of its growth on glycerin agar (see Chapter II).

In many cases of suspected glanders in man, a history of contact with the disease in equines or with glanders cultures will be a useful guide. For the rest, diagnosis will be based on recognition of the specific bacillus by culture or by animal inoculation, or will be decided by serum tests.

Immunization.

Immunity, Natural and Naturally Acquired.

Among the domesticated animals, the ox and the pig, and among small experimental animals, rats and house mice, are immune both to natural and to artificial infection with large doses of bacilli. The sheep also enjoys a considerable degree of natural immunity. As has been mentioned previously, natural resistance is a variable property, even in the equine race. The horse is more resistant than the ass or mule and in nature frequently recovers completely.

So far as naturally acquired immunity is concerned, Galtier's experiments (1881) on dogs showed that local lesions could be produced several times in succession in the same animal. It is known, however, that animals treated in this way are rendered refractory to some degree, since they are capable of resisting amounts of infective material which are fatal to control animals.

In the case of horses certain observations of Nocard (1899) led him to the belief that a natural attack of the disease leaves no immunity. Thus of 2,037 occult cases reacting to the mallein test, 687 subsequently developed clinical lesions and many of the remainder in time ceased to react. In a large proportion of these, nodules were found *post mortem* in which living bacilli could not be demonstrated either by culture or by animal inoculation. Four of the presumably recovered animals were fed with glanders bacilli—the growth from one potato slope being divided among the four animals—with the result that fifteen days later they again reacted to mallein.

Artificial Immunization with B. mallei *or its products.*

Living cultures. M. Nicolle (1906[1]) showed that guinea-pigs could be immunized by amounts of living culture just sufficient to produce obvious infection. Smaller amounts than this produced a lower degree of immunity.

Attenuated cultures. Galtier (1881) believed that by cutaneous passage in dogs the glanders bacillus was attenuated, since asses inoculated with the passage material developed an unusually mild infection. Sacharoff (1893) on similar grounds believed that passage of the organism through cats lowered the virulence for equines. The virulence for the cat was at the same time increased. F. K. Kleine (1903) concluded that inactive strains of *B. mallei*, such as might be useful for immunization, do not exist in nature. Moreover, he was unable to attenuate cultures by means of heat.

Dead cultures. A number of attempts were made by earlier workers to produce immunity by means of cultures killed by heat. It appears that now and again the resistance of certain less susceptible species has been raised by means of cultures killed by heat, but the results have not been uniformly satisfactory.

Little success has been achieved in efforts at vaccinating more susceptible species with dead cultures. Thus, F. K. Kleine (1903) failed to immunize guinea-pigs with cultures heated at 60° C. or with cultures which had been treated with bile. M. Nicolle (1906[1]) demonstrated that guinea-pigs could be vaccinated, though with some difficulty, by repeated injections of dead bacilli. Mohler and Eichhorn (1913) attempted to vaccinate 20 guinea-pigs and 17 horses by means of repeated doses of dried bacilli. The injections led to high agglutination and complement-fixation titres but to no increased resistance to infection. It was found that animals with a complement-fixation titre of 0·02 c.cm. or an agglutination titre of 1 in 5,000 could be readily infected. Schütz and Waldmann (1921) tried to immunize 4 horses by giving each animal 3 injections of bacilli dried *in vacuo* and then heated to 80° C. for an hour. The treatment produced high complement-fixation titres. The animals were subsequently inoculated subcutaneously with $\frac{1}{50,000}$ loopful of living bacilli and 3 out of the 4 developed glanders.

The results obtained by Levy, Blumenthal and Marxer (1907) with dead vaccines are of greater importance. These authors subjected bacilli to solutions of 80·0 per cent. glycerin or 10·0 per cent. urea at varying temperatures. Survival in these solutions depended to some extent upon the density of the emulsion and upon the temperature. For instance, in 80·0 per cent. glycerin the bacilli contained in a suspension of which 4·0 c.cm. contained 0·1 gm. survived for 14 hours at 37° C., whereas in a suspension of which 1·0 c.cm. contained 0·001 gm. the bacilli failed to survive for more than 7½ hours at the same temperature. After complete destruction the bacilli were freed from the suspending fluid by centrifuging, and dried *in vacuo* at a low temperature. The resulting powder, which was known as ' farase ', was found to produce immunity in rabbits and guinea-pigs if given in small doses, though larger amounts were fatal, especially if given intraperitoneally. Horses which had received a single subcutaneous injection of 600·0 mgm. of ' farase ' or two consecutive doses at three weeks interval of 300·0 mgm. were found to develop an immunity lasting for a year. The vaccine caused only slight local and general disturbance and at the same time immune properties developed in the blood.

Experiments on the vaccinating properties of ' farase ' have been carried out by several other observers. Bautz and Machodin (1910) describe an experiment in which 6 foals were vaccinated with ' farase '.

Forty-five days later 4 of the vaccinated animals and 2 controls received subcutaneous injections of $\frac{1}{5000}$ or $\frac{1}{1000}$ loopful of glanders bacilli, while the two remaining vaccinated animals were subjected to natural infection by contact. The two controls developed generalized glanders. Of the 6 vaccinated animals 2 showed abscesses at the site of injection, and 3 showed nasal catarrh which could be ascribed to other causes. Dediulin (1911) immunized with ' farase ' 303 horses which had passed the mallein test, but which were living in infected surroundings. Two injections of 0·2 to 0·4 gm. and 0·4 to 0·8 gm. of ' farase ' were given at an interval of 3 weeks, and 297 horses were left as controls. During the succeeding sixteen months no cases of glanders developed among the vaccinated animals, while among the controls fourteen cases were reported. Four years later, reference was made to this experiment by Marxer (1915), who stated that the vaccinated animals appeared healthy, while cases of glanders were still appearing among the controls. Treatment of glanders in man with ' farase ' gave good results in one case (Fischer, 1920). Minimal amounts only could be used, because doses exceeding 0·5 mgm. were found to cause severe systemic disturbance. Favourable results have also been reported by the same author in cases treated by heat-killed bacilli.

Extracts of B. mallei. Repeated injections of mallein have been tried unsuccessfully for producing active immunity and for curative treatment in equines. Mallein has also been used for the treatment of human glanders, but as with tuberculin caution has to be exercised in the matter of dosage. One instance of chronic glanders is known in which 22·0 c.cm. mallein was injected within nine days. The temperature gradually rose from about 102 to 105° F. on the ninth day, when the patient died. Zieler (1903) recommended that minimal amounts should be injected in the first place to determine whether the patient was hypersensitive. 2·0 to 5·0 mgm. Foth's dried mallein was found to produce considerable disturbance in glanders patients. Bonome (1894) reported that repeated injections of minimal amounts of mallein have a beneficial result in human glanders. Following upon the work of A. Babes (1892) which showed that guinea-pigs could sometimes be immunized by repeated injections of ' morvin '—the dried precipitate obtained from culture filtrates—V. Babes (1894) recommended minimal amounts (0·002 to 0·004 mgm.) of the substance for the treatment of cases in man.

Serum Therapy.

Attempts have been made to produce an immune or hyperimmune serum for preventive or curative purposes. F. K. Kleine (1903) prepared an agglutinating serum of high titre (20,000) by injecting goats with heat-killed bacilli. When given intraperitoneally to guinea-pigs, the serum was found to be incapable of preventing infection by small amounts of living culture inoculated by the same route 10 minutes to 3 hours later. Watson (1923) injected horses with mallein, and produced a serum which was considered to have given useful results in treatment of cases of glanders in man. Noniewicz (1927) has reported a similar curative effect in human cases by the use of a serum prepared by injecting sheep with dead bacilli or with ' farase '.

Chemotherapy.

Numerous attempts have been made at chemotherapy, especially in cases of human glanders, but without any outstanding success. Among the reagents which have been tried are salvarsan, Bayer 205, mercury internally or in the form of inunctions, colloidal silver, iodine, potassium iodide, arsenic, iron and quinine. It is interesting to note that certain of these reagents, e.g. salvarsan and mercury cause the disappearance of complement-fixing properties from the blood, though lesions in the organs can still be found after death.

Antibacterial Measures.

Disinfection.

The glanders bacillus has a low resistance not only to physical agencies such as light, heat and desiccation, but also to chemical disinfectants. Löffler (1886) found that solutions of 3·0 per cent. carbolic acid, 1·0 per cent. potassium permanganate, 1·0 per cent. lysol or 1 in 5,000 mercuric chloride were fatal within 2 to 5 minutes to bacilli from culture which had been dried on silk threads. Cohen (1919) studied the resistance of *B. mallei* to solutions of calcium hypochlorite in the concentrations commonly used for the disinfection of water supplies. *B. coli* was used as a control, owing to its known sensitiveness to this reagent. In order to imitate the conditions as they occur in water troughs, the test medium used consisted of tap water to which a certain amount of vegetable organic matter was added in the shape of stems and leaves. Calcium hypochlorite was then added to 100 c.cm. amounts of the tap water medium to give concentrations of available chlorine ranging from 0·1 to 2·0 parts per million. 0·1 to 1·0 c.cm. emulsion of *B. mallei* was then added and tests for survival made at hourly intervals by plating on glycerin agar. The temperature of exposure was 22° C. In the conditions of the experiments 0·1 part available chlorine per million was ineffective ; a concentration of 2·0 parts available chlorine per million was effective with an overnight exposure. If the seeding with glanders bacilli was light (about 150,000 organisms per c.cm.), this strength was effective within 30 minutes. Thus, *B. mallei* is even more sensitive to chlorine than *B. coli*, and consequently, calcium hypochlorite may be looked upon as reliable for the disinfection of water in horse troughs.

Methods adopted in Civilized Countries for Controlling the Disease in Equines.

Legislation.

Experience has shown that glanders in equines may be brought under control by employing modern methods of diagnosis, and by the slaughter of clinical and occult cases, followed by disinfection of premises.

In Great Britain the disease is scheduled under the Diseases of Animals' Acts and is administered under the Glanders and Farcy Order of 1920. The main provisions of the Order deal with the following points. Notification of diseased or suspected cases in the horse, ass or mule is to be made to the Local Authority. If veterinary enquiry indicates the possibility of the disease existing, notification is made to the Ministry of Agriculture. The Local Authority is empowered to order the destruction of all cases in which the clinical symptoms furnish definite evidence of disease, and of cases which have reacted to mallein. Contact animals are to be detained and with the owner's consent tested with mallein. Other provisions of the Order deal with compensation, destruction of contaminated fodder, litter, &c., disinfection of infected places and disposal of carcasses by deep burial or by cremation.

In France proved cases of glanders are to be immediately destroyed under the Act of 1898, while a Decree dated 1904 gives details of other measures which are applicable. Suspected animals are tested with mallein and reactors destroyed. The premises from which such animals come are declared to be infected and the following regulations come into force:

1. The owner is not permitted to introduce healthy susceptible animals.
2. Contact animals are placed under veterinary supervision for a period of six months counting from the last case diagnosed. Contact animals may be worked under certain restrictions, so long as they remain apparently healthy, but access to common watering-troughs or to other premises is not permitted. They may not be sold except for slaughter. Any animal presenting suspicious symptoms during the period of supervision is to be malleined, and, if reacting, destroyed. On the other hand, the owner may elect to have the whole of the contact animals malleined. Animals which do not react at two tests applied at one month's interval and which after the first test have been placed in disinfected stables, are freed. Latent cases which react are retested every two months, and are not liberated until two tests at sufficient interval have indicated freedom from glanders. The law requires a final disinfection of the premises before restrictions are removed. Other regulations deal with compensation.

In Germany glanders is controlled under the Diseases of Animals Act of 1909. All suspect animals must be slaughtered in which the disease has been diagnosed, either on clinical grounds or following the application of the combined agglutination and complement-fixation test, the ophthalmic mallein test or any other tests approved by the State authorities. Apparently healthy contacts are also to be isolated and placed under veterinary supervision for a period of at least six months. They may be released, however, before this if all the animals are found by clinical and serological examination to be free from suspicion. Other regulations deal with the disinfection of premises and of persons who have handled affected animals, disposal of carcasses, compensation, and the importation of animals affected or suspected to be suffering from contagious disease (Wehrle, 1927).

Control of the Disease in Man.

Persons, such as grooms and others, who by the nature of their occupation are brought into contact with animals suffering from or suspected of glanders should be warned of the possibility of human infection. Abrasions on the hands or arms should be covered and the smallest injury sustained during work should be immediately treated by energetic methods, such as cauterization or the application of strong disinfectants. Laboratory workers should exercise great care when handling known or suspected cultures of *B. mallei* or glanders material, and the possibility of infection by the mouth should not be overlooked.

Human beings affected with glanders are also a source of danger to those about them and should be isolated. Wound dressings should be promptly burnt and bedclothes and garments liable to be soiled by the discharges should be disinfected before being washed. Attendants should wash their hands immediately after dressing wounds and instruments must be carefully sterilized after use.

REFERENCES.

Babes, A., 1892, *Arch. méd. exp.*, **4**, 450.
Babes, V., 1891, *Arch. méd. exp.*, **3**, 619 ; 1894, *Sem. méd. Paris*, **14**, 373.
Basset, J., 1907, *Bull. Soc. cent. Méd. vét.*, **61**, 334.
Baumgarten, 1890, *Lehrbuch der pathologischen Mykologie.*
Bautz, F. & Machodin, S., 1910, *Berl. tierärztl. Wschr.*, **26**, 264.
Blaimschein, F., 1917, *Wien. tierärztl. Mschr.*, **4**, 241.
Bonome, A., 1894, *Deuts. med. Wschr.*, **20**, 725.
Bouchard, Ch., Capitan & Charrin, 1882, *Bull. Acad. Méd. Paris*, 2 s, **11**, 1485; 1883, *ibid.*, 2 s., **12**, 1239.
Brocq-Rousseu, Forgeot, P. & Urbain, A., 1921, *Ann. Inst. Pasteur*, **35**, 879; 1923, *C.R. Soc. Biol.*, Paris, **89**, 1104.
Bulloch, W. & Twort, F. W., 1905, *Zbl. Bakt.*, Abt. I, Orig., **39**, 29.
Cadéac & Malet, 1886, *C.R. Acad. Sci.*, Paris, **103**, 398.
Calmette, A. & de Potter, 1926, *Ann. Inst. Pasteur*, **40**, 353.
Cantacuzène, J. & Riegler, P., 1907, *Ann. Inst. Pasteur*, **21**, 194.
Chauveau, A., 1868, *C.R. Acad. Sci.*, Paris, **56**, 359.
Ciuca, M., 1910, *C.R. Soc. Biol.*, Paris, **68**, 77.
Cohen, B., 1919, *J. Infect. Dis.*, **24**, 51.
Collins, K. R., 1908, *J. Infect. Dis.*, **5**, 401.
Costa, S. & Fayet, A., 1911, *C.R. Soc. Biol.*, Paris, **70**, 147.
Dediulin, A., 1911, *Z. InfektKr.*, **9**, 382.
Drouin & Naudinat, 1914, *Bull. Soc. cent. Méd. vét.*, **68**, 385.
Duval, C. W. & White, P. G., 1907, *J. Exp. Med.*, **9**, 352.
Fayet, 1915, *Bull. Soc. cent. Méd. vét.*, **68**, 516.
Fischer, O., 1920, *Deuts. med. Wschr.*, **46**, 73.
Foth, H., 1892, *Z. Veterinärk.*
Foulerton, A. G. R., 1897, *Lancet*, Lond., i, 1201.
Fröhner, E. & Habersang, 1920, *Mhft. prakt. Tierheilk.*, **31**, 1.
Galtier, V., 1881, *C.R. Acad. Sci.*, Paris, **92**, 303.
Giese, C. & Krüger, H., 1924, *Z. InfektKr.*, **26**, 1.
Helmann, C., 1891, quoted by Wladimiroff, 1913, 1125.
Hunting, W., 1908, *Glanders*, London (H. & W. Brown), p. 14.
Hutyra, F., 1907, *Z. Tiermed.*, **11**, 1.
Hutyra, F. & Marek, J., 1922, *Spezielle Pathologie und Therapie der Haustiere.* VI Aufl., **1** (1926, Eng. Trans.), 804.
Israel, O., 1883, *Berl. klin. Wschr.*, **20**, 55.

Izkowitsch, 1888, quoted by Wladimiroff, 1913, p. 1094.

Kalning, O., 1891, quoted by Wladimiroff, 1913, 1125.

Kitt, 1885, *Jber. Kgl. Central. Thierarzneischule München* (1883–4), Leipz., p. 38.

Kleine, F. K., 1903, *Z. Hyg. InfektKr.*, **44,** 183.

Kondo, S. & Oguni, H., 1918, *Bull. Inst. Pasteur,* **16,** 593.

Konew, D., 1910, *Zbl. Bakt.*, Abt. I, Orig., **55,** 251.

Laboulbène, 1883, *Gaz. Hôp.*, Paris, **56,** 833, 843, 857, 881, 905.

Lanfranchi, 1914, *Mod. Zooiat. Torino,* **25,** 1.

Leclainche, E., 1892, *Rev. Vet. Zootech.*, **49,** 465.

Leclainche, E. & Montané, L., 1893, *Ann. Inst. Pasteur,* **7,** 481.

Levy, E., Blumenthal, F. & Marxer, A., 1907, *Z. InfektKr.*, **3,** 294.

Löffler 1886, *Arb. GesundhAmt.*, Berl., **1,** 141.

Löffler & Schütz, 1882, *Deuts. med. Wschr.*, **8,** 707.

Lührs, E., 1927, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **6,** 1.

M'Fadyean, J., 1893, *J. Comp. Path.*, **6,** 36 ; 1895, *ibid.*, **8,** 50 ; 1896, *ibid.*, **9,** 322 ; 1901, *ibid.*, **14,** 265 ; 1904, *ibid.*, **17,** 295 ; 1905, *ibid.*, **18,** 23.

Martini, 1911, *Z. Hyg. InfektKr.*, **68,** 85.

Marxer, A., 1915, *Arch. wiss. prakt. Tierhlk.*, **41,** 272 ; 1919, *Z. ImmunForsch.*, Tl. I, Orig., **28,** 410.

Meyer, K. F., 1913, *J. Infect. Dis.*, **12,** 170.

Miessner, H., 1912, *Zbl. Bakt.*, Abt. I, Orig., **63,** 482.

Miessner & Trapp, 1909, *Arch. wiss. prakt. Tierhlk.*, **35,** 84.

Mohler, J. R. & Eichhorn, A., 1911, Diagnosis of glanders by complement fixation, *Bull. U.S. Dept. Agric.*, No. 136, p. 19 ; 1913, *Amer. Vet. Rev.*, **44,** 31.

Nicolle, M., 1906^1, *Ann. Inst. Pasteur,* **20,** 625 ; 1906^2, *ibid.*, **20,** 698 ; 1906^3, *ibid.*, **20,** 801.

Nocard, 1894, *Bull. Soc. cent. Méd. vét.*, **48,** 225 & 367 ; 1896, *ibid.*, **50,** 196 ; 1899, *ibid.*, **53,** 502.

Noniewicz, E., 1927, *Bull. Inst. Pasteur,* **25,** 863.

Nowikoff, A., 1895, quoted by Wladimiroff, 1913, 1092.

Oyuela, A. M., 1912, *C.R. Soc. Biol.*, Paris, **72,** 929.

Panisset, L., 1910, *Rev. gén. Méd. vét.*, **15,** 561.

Pfeiler, W., 1909, *Arch. wiss. prakt. Tierhlk.*, **35,** 323 ; 1910, *Z. InfektKr.*, **7,** 328 & 465 ; 1915, *Berl. tierärztl. Wschr.*, **31,** 397 & 411.

Pfeiler, W. & Weber, G., 1912, *Berl. tierärztl. Wschr.*, **28,** 785.

Povitzky, O. R., 1918, *J. Immunol.*, **3,** 463.

Reinhardt, R., 1913, *Z. InfektKr.*, **13,** 295.

Renault & Bouley, 1840, *Rec. Med. vét.*, **17,** 538.

Reverdin, A. & Grumbach, A., 1924, *Ann. Méd.*, **15,** 42.

Robins, G. D., 1906, *Studies from the Royal Victoria Hospital, Montreal,* **2,** No. 1.

Sabolotny, S. S., 1926, *Zbl. Bakt.*, Abt. I, Orig., **98,** 37.

Sacharoff, 1893, quoted by Marxer, 1915.

Schnürer, J., 1905, *Zbl. Bakt.*, Abt. I, Orig., **39,** 180 ; 1910, *Berl. tierärztl. Wschr.*, **26,** 261 ; 1911, *Z. InfektKr.*, **10,** 321 & 408.

Schoening, H., 1917, *J. Agric. Res.*, **11,** 65.

Schreiber, O. & Stickdorn, W., 1915, *Berl. tierärztl. Wschr.*, **31,** 553.

Schütz, 1894, *Arch. wiss. prakt. Tierhlk.*, **20,** 425.

Schütz & Miessner, 1905, *Arch. wiss. prakt. Tierhlk.*, **31,** 353.

Schütz & Schubert, 1909, *Arch. wiss. prakt. Tierhlk.*, **35,** 49.

Schütz & Waldmann, O., 1921, *Arch. wiss. prakt. Tierhlk.*, **46,** 172.

Stanton, A. T. & Fletcher, W., 1925, *J. Hyg.*, Camb., **23,** 347.

Straus, I., 1889, *Arch. méd. exp.*, **1,** 460.

Struck, 1882, *Deuts. med. Wschr.*, **8,** 707.

Südmersen, H. J. & Glenny, A. T., 1908, *J. Hyg.*, Camb., **8,** 14.

Tatray, quoted by Hutyra & Marek, 1922, **1,** 841.

Tedeschi, 1893, quoted by J. H. Wright, 1896, *J. Exp. Med.*, **1,** 577.

Turró, R., 1908, *C.R. Soc. Biol.*, Paris, **64,** 130.

Vallée, H., 1907^1, *Bull. Soc. cent. Méd. vét.*, **61,** 308 ; 1907^2, *ibid.*, **61,** 359.

Vehse, H., 1920, *Mhft. prakt. Tierheilk.*, **31,** 363.

Wade, E. M., 1913, *J. Infect. Dis.*, **12,** 7.

WALDENBURG, 1869, *Die Tuberculose, die Lungenschwindsucht und Scrofulose,* Berl., 186–198.
WALDMANN, O., 1914, *Arch. wiss. prakt. Tierhlk.*, **40,** 382 ; 1916, *ibid.*, **42,** 194.
WATSON, E. A., 1923, *J. Amer. Vet. Med. Ass.*, **64,** 146.
WATSON, E. A. & HEATH, L. M., 1922, *J. Amer. Vet. Med. Ass.*, **61,** 503.
WEHRLE, E., 1927, Personal communication.
WEICHSELBAUM, A., 1885, *Wien. med. Wschr.*, **25,** 665, 700, 737, 765.
WHITMORE, A., 1913, *J. Hyg.*, Camb., **13,** 1.
WLADIMIROFF, A., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. v. Wassermann, 2nd ed., Jena, **5,** 1063.
ZIELER, K., 1903, *Z. Hyg. InfektKr.*, **45,** 309.
ZURKAN, J., 1911, *Z. InfektKr.*, **10,** 473.

CHAPTER II. MELIOIDOSIS.

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History and Geographical Distribution.

MELIOIDOSIS is a disease which closely resembles glanders. It was first described by Whitmore and Krishnaswami (1912) in the bodies of vagrants brought to the public mortuary by the Police in Rangoon. The Federated Malay States was the next country in which it was recognized. It appeared in 1913 (Fletcher, 1919), not, at first, as a disease of man, but in the form of a severe epizootic among the guinea-pigs and rabbits in the laboratory at Kuala Lumpur. The connection of the causative organism with Whitmore's bacillus, or with a disease of man, was not suspected at the time, though it was noticed that the lesions were like those of glanders. In 1917 Stanton discovered human cases in Kuala Lumpur. He identified the bacillus, and reproduced the disease in a number of different animals, by feeding and by the inoculation of cultures.

Melioidosis was recognized nowhere outside Burma and British Malaya until 1925, when a case was diagnosed by Pons and Advier (1927) in Indo-China. In the following year Vielle, Morin and Massias (1926) reported the occurrence of a second case in the same country, and in 1927 Denny and Nicholls reported another in Ceylon.

Only 65 cases have been recognized, up to the present time, all in the countries mentioned. The victims, with three exceptions, were all males. Three Europeans were affected. It is, however, highly improbable that melioidosis is limited to the few places where its presence has been detected; its symptoms are so varied and obscure that it is seldom diagnosed until after death. Even when laboratory facilities are available, the disease may escape recognition by those who are unfamiliar with it, because the bacillus grows so profusely that it has been mistaken for a contamination.

Kuala Lumpur is the only place where melioidosis is known to attack animals. It has been recognized there in guinea-pigs, rabbits, rats, cats, dogs, and, in one instance, in a horse; but it is rare even in rats, which are probably the natural hosts of the bacillus.

Bacillus whitmori.

Whitmore (1913) identified the causative agent of melioidosis as a bacillus which he called *Bacillus pseudo-mallei*. This name had been employed before for another organism, and, as it was therefore invalid, Stanton and Fletcher (1921) proposed the name *Bacillus whitmori*, which has since been adopted. *B. whitmori* is an organism which it is difficult to classify. It is more nearly related to *B. mallei* than to any other pathogenic organism, and therefore it may be placed with it in the *Pfeifferella* group, though its motility, and its power of actively fermenting carbohydrates when first isolated, make its position somewhat anomalous.

MORPHOLOGY AND STAINING REACTIONS.

Bacillus whitmori, as it is found in the infected tissues and discharges, is a delicate rod with rounded ends which stain more deeply than the middle portion ; this gives it a superficial likeness to *B. pestis*, for which, indeed, it has been mistaken on more than one occasion. It is, however, more rod-shaped, and its straight sides, clear middle, and darkly stained ends give it something of the appearance of a closed safety-pin. It is Gram-negative, and stains readily with all the ordinary dyes. Leishman's stain is particularly useful for demonstrating the bipolar staining, but weak carbol-fuchsine or carbol-thionin act almost as well. The bipolar staining is less pronounced in films made from cultures than in those made direct from the tissues. The bacilli are occasionally very numerous in the tissues and discharges, but as a rule they are scanty. In films, they are found, for the most part, scattered amongst the cells, singly and in pairs.

The organisms in 24-hour broth cultures of recently isolated strains are stouter than the forms seen in the tissues, and measure about 4μ long by 2μ broad. They are mostly in pairs, and there are sometimes a few large elements among them, 15 or even 20μ in length. As the cultures age, the bacilli become shorter, and after three weeks they assume a minute, coccoid form. The individual organisms in young agar cultures are very small, and, on an average, measure only 2 by 1·5μ. Long involution forms grow in broth which contains 1·5 per cent. of sodium chloride ; they are granular elements, 20μ or more in length.

Young cultures are actively motile.

CULTIVATION ON ARTIFICIAL MEDIA.

B. whitmori is easily grown direct from the infected tissues, on all the common laboratory media. It grows profusely at 37° C., and less actively at lower temperatures down to about 20° C. It can be maintained on ordinary agar at laboratory temperatures, in the tropics, but it is rather sensitive to cold when first brought to a temperate climate. A reaction of about pH 7 is most favourable to its growth, but it is not very sensitive in this respect. It is an obligatory aerobe.

One of the most characteristic features of *B. whitmori* is its growth on 5 per cent. glycerin-agar. When this medium is inoculated with an exudate containing the bacilli, small colonies appear in 24 hours which display a curious iridescent sheen when examined with a hand lens. Growth is rapid during the next 24 hours, and, in a short time, the surface of the colonies becomes wrinkled like the surface of a mould. When glycerin-agar slants are inoculated with a loopful of culture, a characteristic wrinkling and corrugation appears after 48 hours, at the bottom and along the edges of the growth, and by the end of a week the culture is heaped up and rugose like an old culture of tubercle bacilli. The early growth on ordinary agar, without glycerin, is white and opaque like a streak of white paint; there is little or no corrugation, unless the strain has been isolated very recently from a case of melioidosis.

A pellicle is formed on the surface of liquid media, which becomes tough and wrinkled in the course of 4 or 5 days. The organism grows well on potato, but without displaying any phenomena of diagnostic importance. The corrugated form grows as a dirty white streak; the mucoid form, which will be discussed presently, is slimy and yellow, like a young culture of the glanders bacillus.

B. whitmori grows in two forms on glycerin-agar; in addition to the more common corrugated form, a slimy, mucoid variety, with some resemblance to *B. mallei*, has been isolated on several occasions (Stanton and Fletcher, 1924). Agglutination, absorption and complement-fixation tests show that the two forms are serologically identical; moreover, if a mucoid colony be inoculated into broth, incubated for a few days, and then plated out, a number of corrugated colonies will be found mixed with the mucoids from which they have taken origin. Colonies of both types have been isolated from the same specimen of pus collected from an abscess, and, occasionally, corrugated forms have been grown from one of a patient's organs and mucoids from another. Freshly isolated, highly virulent strains are usually very corrugated.

The chromogenic power of *B. whitmori*, like that of *B. mallei*, varies with different strains. Glycerin-agar cultures are at first white or cream-coloured, but, in a week or 10 days, they become tinged with brown. One strain, isolated from a rat, has a pinkish tinge; another, from a human case, produces a saffron colour in peptone-water. Mucoid strains give a light yellow growth on potato. All cultures of *B. whitmori* have a mouldy, earthy smell.

BIOCHEMICAL REACTIONS.

B. whitmori liquefies gelatin; the liquefaction begins on the fourth or fifth day at a temperature of 20° C., but at 37° C. growth is much faster and the process is complete in 4 days. It forms acid in many carbohydrate media, but gas in none. Acid is produced within about 4 days in lactose, saccharose, glucose, mannitol, dulcitol, maltose and dextrin. The surface of the peptone-water containing the carbohydrate becomes pink in about 36 hours, when Andrade's indicator is employed, and the colour soon spreads through the medium, reaching the Durham's fermentation tube last of all. Bleaching of the indicator commences on the fourth or fifth day and proceeds in the same order, being complete by the tenth day. The fermentation reactions of the bacillus are more vigorous when it is first isolated; they become enfeebled with repeated subculture, except as regards glucose, which is fermented by strains that have been grown in the laboratory for years. Litmus milk, inoculated with *B. whitmori*, becomes acid in 3 days; in 5, it separates into a fatty curd on the surface, an intermediate layer of dirty, brown whey, and a granular buff-coloured deposit at the bottom of the tube.

B. whitmori does not form indole in peptone-water. It is alkaligenic in broth cultures, and a reaction of pH 7 becomes pH 8 in about 3 weeks. Brown, Duncan and Henry (1924) have shown that *B. whitmori* grows well in peptone-water containing 1 per cent. of sodium fumarate and decomposes the salt. When it is grown in broth containing nitrates it reduces them to nitrites. It does not hydrolyse starch, nor does it produce hydrogen sulphide when examined by Kligler's method.

PERSISTENCE AND CARRIERS.

B. whitmori seldom lives long in its human host, it usually kills its victim too quickly, but the only two cases which are known to have survived for more than a few weeks harboured the organism for many months in the discharge from sinuses connected with the bones of the legs and feet. Similarly, in the rare instance of a guinea-pig which recovered after subcutaneous inoculation, *B. whitmori* was repeatedly found in the pus from a sinus which took several months to heal. Melioidosis runs a comparatively slow course in rats, during which they may act as active disseminators of infection.

DISTRIBUTION AND RESISTANCE OUTSIDE THE BODY.

B. whitmori has great powers of resistance outside the body, in a tropical climate; but it has been found apart from its animal host, in nature, on only one occasion. In this instance it was isolated from green vegetables kept on the floor of an animal house, which had probably been contaminated by the excreta of wild rats infected with melioidosis. A further search for the organism in water, soil and foodstuffs, may prove successful, because *B. whitmori* is very resistant to drying and to destruction by contamination with other organisms. It will survive for as long as nine days on pieces of filter-paper moistened with a broth culture and kept in a vacuum desiccator. Recently isolated cultures have been mixed with fresh soil from a vegetable garden and kept in a desiccator at room temperature (26 to 27° C.) for 27 days; at the end of that time virulent *B. whitmori* were still present, and the disease was reproduced in guinea-pigs by blowing a little of the dust into their nostrils.

B. whitmori can exist for a long time in water and excrement. Recently isolated cultures, mixed with tap water or drinking water, or even with dirty water from a roadside ditch, may survive for as long as 44 days at room temperature. It can also live for a long time in fæces: a strain obtained 48 hours previously from a human case of melioidosis was mixed with fæces and salt-solution, and virulent bacilli could be isolated from the mixture 27 days later. It survives for about 17 days in urine. It has been isolated as late as the eighth day from the putrid and maggoty cadaver of a guinea-pig.

B. whitmori is easily killed by heat. A temperature of 56° C. will kill, within 10 minutes, a suspension containing 4,000 bacilli in 1 c.cm. Freshly isolated cultures are not very resistant to low temperatures; they die in 2 or 3 weeks if they are kept at a temperature of 4° C. The bacillus is sensitive to the action of disinfectants, and is killed in less than 24 hours by either 1 per cent. phenol, at a temperature of 37° C., or by 0·1 per cent. formalin at a temperature of 4° C.

THE INOCULATION OF ANIMALS.

Melioidosis has been produced experimentally in guinea-pigs, rats, mice, cats, dogs, sheep, goats and monkeys. Guinea-pigs inoculated subcutaneously with large doses of virulent cultures of *B. whitmori* die from septicæmia in less than 24 hours. The inoculation of a smaller quantity of culture, or of an exudate containing only a few bacilli, is always followed by a pronounced local reaction in the form of a hard swelling, consisting of coagulated lymph and œdematous necrotic tissue in which the organism can be readily demonstrated. A small slough appears on about the third day, in the middle of the swelling, at the site of the needle puncture; this enlarges until a crater-like ulcer is formed, with ragged undermined edges and a yellowish slough in the centre. The infection spreads at first by the lymphatics, which become thickened and nodular, and the nearest lymphatic glands enlarge and suppurate. Some of the bacilli are carried away by the blood-stream and lodge in the capillaries of the spleen, liver, lungs and other parts of the body, where they originate pyæmic foci. Septicæmia supervenes, and the animal dies about three weeks after the inoculation.

When the inoculation is made into the skin of the abdomen, peritonitis often results from extension through the abdominal wall; a little fluid is found in the peritoneal cavity, and the omentum, which is rolled up and covered with lymph, contains small suppurating caseous nodules. Guinea-pigs are so susceptible that scarification of the skin, or a mere prick with a needle dipped in a virulent culture of *B. whitmori*, is sufficient to cause a fatal infection. In a few rare instances, an inoculated guinea-pig has recovered after healing of the primary lesion and prolonged suppuration of the lymphatic glands. The inoculation of a few bacilli into the peritoneal cavity of a guinea-pig causes peritonitis with scattered melioidosis nodules in the omentum and viscera. If the guinea-pig is a male, an enlargement of the testes, similar to Straus's reaction in glanders, appears within 48 hours. The tunica vaginalis becomes thickened and full of small suppurating nodules; subsequently it is converted into a caseous mass adherent to the skin. Owing to the great pathogenicity of the organism, the guinea-pig will die from septicæmia before this reaction occurs, if more than about 0·01 c.cm. of an 18-hour broth culture is injected.

Guinea-pigs are readily infected by ingestion and inhalation, and the natural features of the disease are produced if a culture is sprinkled on the food, or a little is painted on the mucous membrane of the nose. Within 3 or 4 days the eyes and nose are moist and dirty with a watery discharge which soon becomes thick and milky, the breathing is obstructed, and the animal dies in a week or 10 days with all the signs of the disease as it occurs during an epizootic.

Rabbits are as susceptible as guinea-pigs, but wild rats (*M. griseiventer* and *M. decumanus*) are more resistant. The subcutaneous inoculation of a rat is not followed by a pronounced local reaction, and it will often live for 30 days after the injection of a dose which would kill a guinea-pig in a week. Occasionally, however, a very virulent culture will cause the death of a rat from septicæmia in 24 hours, with intense subcutaneous injection associated with hæmorrhagic enlargement of the cervical and axillary glands—a condition closely resembling that seen in a rat dead from plague. The natural disease in rats is characterized by massive caseation of the lungs ; sometimes there are large nodules in the spleen like those in the spleen of a rat with resolving plague. A condition exactly similar to this can be produced by feeding rats on cultures. The bacilli are scanty in films made from the blood and spleen of rats, but they are often very numerous in the caseous areas in the lungs, lying almost entirely within large cells.

A few experiments have been made with monkeys (*Macacus cynomolgus*). They are more resistant than rodents and, in some instances, they have ingested large quantities of virulent cultures without result ; in other cases a fatal infection has followed, with caseous nodules in the lungs and other viscera.

Horses and pigs are unaffected by the ingestion of virulent cultures, and attempts to infect them by intravenous and intranasal injections have proved unsuccessful. Subcutaneous inoculation produces only a localized suppuration, which disappears in a week or two without any trace of generalization. The case of a horse, in which a transient nasal affection occurred, will be mentioned under the heading of pathology. Domestic fowls are immune.

Analysis of Pathogenic Action.

The pathogenic action of *B. whitmori* has not been thoroughly analysed. It does not produce exotoxins or hæmolysins in broth cultures. The inoculation of filtrates has no effect. The subcutaneous inoculation of heat-killed cultures causes very little local or general reaction ; and the injection of old broth cultures, after boiling, produces only an indurated red patch which disappears in a few days.

Maintenance of Virulence in Cultures.

All the strains of *B. whitmori* which have been examined are highly pathogenic for guinea-pigs and rabbits. Those isolated during a laboratory epizootic in Kuala Lumpur were the most highly virulent for rodents. The organism retains its pathogenicity over long periods during cultivation on artificial media. The oldest strain in existence, after being grown on glycerin-agar for eight years at a temperature of about 26·5° C., was compared with one which had been recently isolated, by means of inoculating guinea-pigs : the new strain killed them in 4 to 10 days, while the old strain killed them in 2 or 3 weeks.

THE RELATIONSHIP OF *B. WHITMORI* TO *B. MALLEI* AND TO A SAPROPHYTE OF RATS.

Melioidosis resembles glanders very closely. Though the former usually runs a more rapid course, the symptoms of the two diseases are similar. It is very difficult to distinguish the lesions of the one from those of the other, either with the naked eye or with the microscope. The causative organisms have a similar morphology ; young cultures of the mucoid form of *B. whitmori* are indistinguishable, by inspection alone, from *B. mallei*, though the growth of the former is much more rapid. Both organisms produce Straus's reaction in guinea-pigs.

B. whitmori differs from *B. mallei* in the following particulars : it is actively motile ; it forms a corrugated growth on glycerin-agar, an opaque white growth on ordinary agar, and a continuous pellicle on the surface of broth ; it grows more rapidly than *B. mallei*, it attacks a larger range of carbohydrates when first isolated ; it liquefies gelatin more quickly ; and it decomposes sodium fumarate.

Stanton and Fletcher (1925) have made a serological comparison of fourteen strains of *B. whitmori* and five strains of *B. mallei*. The strains of *B. mallei* comprised one from Java, one from Muktesar in India, and three from the National Collection of Type Cultures in London. Agglutination, absorption, and complement-fixation tests showed that the cultures of *B. whitmori* formed a homogeneous group ; but the five strains of *B. mallei* were sharply divided into two subgroups by their serological reactions ; one subgroup included the Muktesar and Java strains, the other comprised the three strains from the National Collection. *B. whitmori* was found to be closely related to the first subgroup, particularly to the strain from Muktesar. The strains of *B. mallei* from the National Collection, on the contrary, showed but little affinity with *B. whitmori*. It is possible that further light may be thrown on the relation of *B. whitmori* to the different groups of the glanders bacillus, by the employment of Felix's method of qualitative receptor analysis.

A bacillus which resembles *B. whitmori* in several respects has been isolated, on several occasions, from healthy wild rats in Kuala Lumpur. The bacillus in question is a Gram-negative, motile organism which is morphologically indistinguishable from *B. whitmori* ; it has the same action on carbohydrates, and its growth on glycerin-agar is very similar ; on the other hand, it is not agglutinated by immune sera prepared with *B. whitmori*, nor is it pathogenic for rats, rabbits, or guinea-pigs.

Pathology.

The characteristic lesion of melioidosis is a small caseous nodule which commences as a collection of polynuclear cells surrounded by a zone of congestion. When these nodules have become large enough to be visible to the naked eye, they consist of a mass of necrotic caseous material containing a great number of chromatin fragments which are the tattered nuclei of dead cells; this agglomeration of darkly staining, irregular particles gives a characteristic appearance to the melioidosis nodule. Since the distribution of the lesions and the course of the disease are not altogether the same in man and animals, they are considered separately.

PATHOLOGY IN MAN.

Melioidosis nodules have been found in almost every part of the body except the brain. In some cases they coalesce to form large areas of caseation; in others they break down into abscesses. Wherever they are found, *B. whitmori* can be cultivated from them. The lungs are nearly always affected, and, when the disease is not far advanced, the nodules can be felt like shot within their substance. When the process has gone further, the nodules coalesce and the lungs are filled with caseous masses; nodules in the lungs rarely break down to form large abscesses. The spleen is enlarged in melioidosis; it frequently contains nodules, and sometimes small abscesses filled with creamy pus. Nodules in the liver are particularly prone to suppurate, producing multiple, round, sharply-demarcated abscesses, full of thick, greenish-white pus. The kidney is sometimes the seat of multiple abscesses, especially in women, and, occasionally, the bladder is affected. In such cases *B. whitmori* is present in the urine. Abscesses may also be found in the subcutaneous tissue, in the muscles and in connection with the bones. Small superficial ulcers have been found in the large intestines in a few cases, but *B. whitmori* has never been found in the gastro-intestinal tract of man, though it has been cultivated from the intestine of a rabbit in one instance, and, in another, from the stomach of a rat which had a nodule in its stomach wall. The organism has never been isolated from the fæces of men or animals during life. The gall-bladder is sometimes affected, and *B. whitmori* has been cultivated from the bile. In the late septicæmic stage of the disease it can be cultivated from the spleen and the peripheral blood.

PATHOLOGY IN ANIMALS.

Melioidosis is primarily a disease of rodents. It occurs naturally in rats (*M. griseiventer*), guinea-pigs, and rabbits, and, occasionally, in cats and dogs which hunt rodents.

The first symptoms of the epizootic disease, in rabbits and guinea-pigs, is a white, milky discharge from the eyes and nose, which contains *B. whitmori*; this is followed in a few days by dyspnœa and death. The sporadic form is much less acute, there is rarely any nasal discharge, but the animal gets thin and out of condition, its breathing becomes rapid and it dies at the end of 2 or 3 weeks.

The condition found after death depends largely upon the duration of the disease. In animals which have died quickly from acute septicæmia, the only visible signs apart from the milky discharge are congestion of the nasal mucosa, trachea and lungs, with a few yellow miliary nodules on the nasal septum, and an enlarged spleen. The spleen often has a granular surface. The bladder is sometimes enormously distended. When the course of the disease is longer, the lungs are full of minute caseous nodules, like miliary tubercules, which consist of a bronchiole and its surrounding alveoli filled with nuclear debris. In animals which have lived still longer, the lungs are consolidated, the bronchi are choked with detached epithelium, there are small scattered hæmorrhages, and in places all alveolar structure is lost.

The disease runs a less rapid course in rats. There is no milky discharge from the eyes and nose at the onset, but the animal refuses food and loses flesh ; it sits huddled up and panting in a corner of its cage and dies from septicæmia in 3 or 4 weeks. Miliary nodules like those which occur in the lungs of guinea-pigs are seldom found in rats which have died from melioidosis, because, in them, the disease lasts longer and the nodules spread and coalesce until the lungs are converted into a caseous mass which fills the thorax and surrounds the heart. The spleen is always enlarged.

Peritoneal lesions are often found in rodents which have died from melioidosis ; in some cases the peritoneum is covered with small tubercles, resembling those of miliary tuberculosis, and the omentum is rolled up and studded with yellow, caseous nodules. The liver is affected in about 10 per cent., and occasionally there are nodules in the kidney. The testes of male guinea-pigs are sometimes adherent to the scrotum, and the epididymis and tunica vaginalis are filled with caseous nodules. The lymphatic glands in the region of the jaw, neck, and axillæ may be enlarged and hæmorrhagic, with suppurating nodules in their substance. Lesions in the heart are comparatively rare ; occasionally there are yellow nodules, consisting of fibrin and nuclear debris, adhering to the endocardium ; sometimes there is fluid in the pericardium, or the sac may be filled with caseous material.

Fatal melioidosis in a horse is unknown ; Stanton, Fletcher and Symonds (1927) have described, however, a transient nasal discharge, in a horse, which contained *B. whitmori.*

DISSEMINATION AND METHOD OF TRANSFERENCE.

It seems strange that melioidosis is not more common when one considers that *B. whitmori* is able to survive for a comparatively long time outside the body in dry earth, water, fæces, and urine. Man is probably not readily susceptible, otherwise cases of melioidosis would surely have occurred in laboratories where living cultures have been extensively handled, or among the Asiatic attendants who have looked after hundreds of infected animals.

There is no evidence of direct infection from man to man. As *B. whitmori* has been found in the sputum, the urine, and the discharge from superficial lesions, one would expect it to pass readily from the sick to the healthy, but this does not happen. Patients suffering from melioidosis have not been segregated; they have been treated in the general wards of hospitals, yet they have not communicated the disease to others. The way in which man contracts melioidosis is a problem as yet unsolved. The disease resembles glanders very closely, but there are few horses in the places where melioidosis occurs, and, in those cases where it has been possible to investigate the point, there has been no opportunity of infection from these animals.

Only one case has been recorded in which there was evidence of a primary lesion such as results from the subcutaneous inoculation of guinea-pigs; in this instance (Fletcher, 1926) *B. whitmori* was isolated from an ulcer on the leg, a bubo in the groin, and from the suppurating iliac glands on the same side.

Stanton and Fletcher (1924) have seen a number of cases in which the onset of the disease was marked by severe gastro-intestinal symptoms, and they agree with Whitmore (1913) that infection usually occurs by way of the alimentary tract. They suggest that this takes place through the consumption of food which has been contaminated by the excreta of infected rodents.

Practical Diagnosis.

Melioidosis presents no cardinal symptoms by which it can be recognized, and a trustworthy diagnosis can be made only by cultivation of the causative organism. In practice, a correct diagnosis has been made during the life of the patient, in only 7 of the 65 cases of which records are available. In three cases *B. whitmori* was cultivated from the blood; in one from the urine; in one from a pustular eruption; in another from an abscess in the parotid; and, in the seventh, from an abscess in the leg.

The best way to make a diagnosis in places which are distant from a laboratory is to inoculate guinea-pigs with blood or pus and send them to the nearest laboratory for examination. After the death of the patient, guinea-pigs may be inoculated with the spleen pulp and with material from any lesions which are found. If the material is putrid the inoculation can be made by painting it on the nasal mucosa or by scarification. Pieces of the spleen, and of any organs which contain lesions, should be sent to the laboratory; some, in the fresh state, for bacteriological examination; and some, in a fixing fluid, for histological examination.

B. whitmori is seldom very numerous in films made from pus and exudates, but occasionally a tentative diagnosis based on the examination of a film has been confirmed by cultivation.

Immunization.

Agglutinating sera of high titre are easily prepared by the intravenous inoculation of rabbits with killed cultures ; but a serum obtained in this way has little protective power. If it is inoculated into a guinea-pig, 24 hours before an experimental inoculation of *B. whitmori*, the onset of septicæmia is postponed and the animal lives a few days longer than the controls ; but the serum has no effect if it is inoculated on the day after the bacilli. The repeated inoculation of killed cultures confers little or no immunity on laboratory animals, nor has an autogenous vaccine any power to stay the disease in them.

B. whitmori has been employed as a vaccine in two cases of chronic melioidosis. The two patients were the only cases which have been known to recover, but the vaccines were not administered until the men had survived for three and for eight months respectively.

REFERENCES.

Brown, H. C., Duncan, J. T. & Henry, T. A., 1924, *J. Hyg.*, Camb., **23**, 1.

Denny, C. R. & Nicholls, L., 1927, *Ceylon J. Sci.*, Sect. D, **2**, 37.

Fletcher, W., 1919, *Ann. Rep. Inst. Med. Research, Kuala Lumpur ;* 1926, *ibid.*

Pons, R. & Advier, M., 1927, *J. Hyg.*, Camb., **26**, 28.

Stanton, A. T., 1917, *Studies Inst. Med. Research, Kuala Lumpur*, No. 14.

Stanton, A. T. & Fletcher, W., 1921, *Trans. 4th Cong. Far East Assoc. Trop. Med. Hyg.*, **2**, 196 ; 1924, *Bull. Inst. Med. Research, Kuala Lumpur*, No. 5 of 1924 ; 1925, *J. Hyg.*, Camb., **23**, 347.

Stanton, A. T., Fletcher, W. & Symonds, S. L., 1927, *J. Hyg.*, Camb., **26**, 33.

Vielle, A., Morin, H. G. S. & Massias, C., 1926, *Bull. Soc. Med. Chir. Indo-Chine*, No. 9, Sept.

Whitmore, A., 1913, *J. Hyg.*, Camb., **13**, 1.

Whitmore, A. & Krishnaswami, C. S., 1912, *Ind. Med. Gaz.*, **47**, 262.

CHAPTER III. *CORYNEBACTERIUM DIPHTHERIÆ* AND DIPHTHEROID ORGANISMS.

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With a Section

By W. Bulloch (London Hospital).

Introduction.

I desire to express my great indebtedness in the preparation of this Chapter to the Monograph *Diphtheria* issued by the Medical Research Council.* My task has been indeed in large measure to epitomize that work, with the addition of matter that has appeared since it was published in 1923. The sequence of contents adopted in *Diphtheria* has been followed, and considerable portions of the text have been incorporated in this Chapter with little alteration.

The subjects of 'toxin-antitoxin reaction' and 'preparation and standardization of diphtheria antitoxin' are dealt with in another volume (Vol. VI) and are, therefore, omitted from the following account.

The History of Diphtheria.

By W. Bulloch.

Up till the beginning of the nineteenth century great confusion prevailed with regard to forms of disease which had a predilection for affecting the upper respiratory passages. Out of the chaos P. F. Bretonneau (1826), the famous clinician of Tours, separated a definite type of throat distemper which he called by the name of diphtherite, since modified to diphtheria. He regarded this condition as a specific inflammation associated anatomically with the production of a pellicle or false membrane on the affected parts. His views, correct as we now know them to be, were not at once accepted, and further confusion was introduced by the separation of two anatomical types of inflammation, which Virchow (1847) characterized as 'croupous' and 'diphtheritic'. Recourse was had to the experimental reproduction of the disease, and in this branch important advances were made by Oertel (1871). The great dissemination of the disease diphtheria about the middle of last century may be regarded as the reason for renewed study, and attempts were made to discover the cause of the condition.

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* By F. W. Andrewes, W. Bulloch, S. R. Douglas, G. Dreyer and A. D. Gardner, P. Fildes, J. C. G. Ledingham, C. G. L. Wolf (London: H.M. Stationery Office, 1923).

At first these efforts were carried out with very imperfect technique and in consequence yield unsatisfactory results. The real bacteriology of diphtheria begins with Klebs (1883) who, discarding his early idea that it was caused by a pleomorphic fungus, now asserted that it was due to a bacillus which he was able to demonstrate in the false membranes in virtue of its great affinity for methylene blue. His research was confined to microscopic examination. At the same time the disease was subjected to a thorough study by F. Loeffler (1884); whose publication is a classic in the bacteriological literature. Starting from the Bretonneau conception that diphtheria is a specific disease, Loeffler realized that the virus should be sought in the lesions, and for this purpose he made an exhaustive microscopic study of 22 cases of pure diphtheria and of 5 of diphtheria complicating scarlet fever. In the latter group the dominant micro-organisms were found to be streptococci, whereas in the true diphtheria cases Loeffler found a second species of microbe, which he identified as the bacilli described by Klebs. He next proceeded to isolate these and was successful by using a medium consisting of a mixture of glucose, veal broth and blood-serum, the whole being finally stiffened by heat. This medium, known as Loeffler's blood-serum, is in habitual use to-day. He gave a very accurate account of the bacilli as they appear in pure cultures, and he inoculated these pure cultures into a large series of animals, and showed that they are pathogenic and produce very definite and constant lesions. Loeffler also found diphtheria bacilli in a virulent state in one normal child out of a series of twenty examined. He was unable to produce diphtheria paralysis, and it was for this reason that he was guarded in his conclusions regarding the ætiological significance of the bacillus. It was not until a year or two after his publication that confirmation of his observations began to appear, and an extension of his work resulted through the important memoirs of Roux and Yersin (1888–90), who not only produced diphtheria paralysis experimentally, but also discovered diphtheria toxin—the principal product through which the diphtheria agent operates. They also suggested and recommended the bacteriological diagnosis of diphtheria by the demonstration of the characteristic bacillus.

Striking results rapidly followed, particularly in connection with the immunity to diphtheria toxin, and these culminated in the famous publication of Behring (1890, 11th Dec.), announcing the fact that immunity to diphtheria is correlated with certain antitoxic substances which specifically neutralize diphtheria toxin. Serum therapy was thus made possible and was soon put into action. The first diphtheria patient treated by antitoxic serum was a child in v. Bergmann's clinic in Berlin. The date was Christmas night 1891 (Wernicke, 1913). A remarkable impulse to serum therapy resulted from the work of Roux and L. Martin (1894), and the method soon became universal. The standardization of diphtheria toxin and antitoxin was worked out carefully, and after great labour, by Ehrlich, his principal communication dating from 1897. From that time onwards an enormous number of researches in every branch of the diphtheria question was published, but nothing further of a fundamental nature appeared until the publication of the method of diagnosis connected with the name of B. Schick (1908), and resulting from it the method of active immunization by toxin-antitoxin mixtures. This was started by Theobald Smith (1907) and Behring (1913), and was practised on a very large scale first in America through the activity of W. H. Park (1913) and his co-workers.

All this knowledge is considered in great detail in the work *Diphtheria, its Bacteriology, Pathology, and Immunology*, published by the Medical Research Council, 1923, which should be consulted as the most complete account extant. W. B.

Morphology and Cultivation of Corynebacterium diphtheriæ.

INTRODUCTION.

Certain natural groups of species having the value of genera exist among the bacteria, and the diphtheria bacillus belongs to a group which is as well defined as any, and is the most important species in this group. The other members of the group are commonly designated ' diphtheroid ' bacilli or organisms from their morphological resemblance to the diphtheria bacillus. In an endeavour to improve the systematic nomenclature of bacteria, Lehmann and Neumann (1896) suggested the generic name ' Corynebacterium ' (Gr. κορύνη = a club) for the diphtheria group and this name has been generally accepted. In these pages, therefore, while continuing to employ the term ' diphtheria bacillus ' colloquially, ' *Corynebacterium diphtheriæ* ' will be adopted as the scientific name of the organism. The organism is also frequently termed the ' Klebs-Loeffler bacillus ', after its discoverers.

The Corynebacteria are non-sporing bacilli, destitute of flagella, and non-motile. They tend to show clubbing at one or both ends and this irregularity in shape is usually associated with a segmented appearance of the protoplasm, in virtue of which the rods when stained appear transversely banded. Further evidence of differentiation within the cell is furnished by the occurrence, in most species, of peculiar granules, showing metachromatic staining, and retaining the stain in the presence of acetic acid. Neither clubbed forms nor metachromatic granules are altogether confined to this genus, but they are eminently characteristic of it.

No member of the genus is acid-fast, but all are in some degree positive to Gram's stain, some strongly so, others, including *C. diphtheriæ* itself, having a tendency to become decolorized with prolonged washing in alcohol. They are mostly aerobic organisms, but some will grow anaerobically, while others flourish best under micro-aerophilic conditions. When sugars are fermented there is never any production of gas. Most of the species fail to liquefy gelatin, but a few do so. Apart from *C. diphtheriæ* the members of the genus exhibit little pathogenic power ; none of them possesses any special resistance against heat or chemical disinfectant agents.

MORPHOLOGY OF *C. DIPHTHERIÆ*.

General Morphological Characters.

In its ordinary form the diphtheria bacillus appears as a straight or slightly curved rod, measuring some 3 to 5μ in length and 0·5μ or a little more in width. In any culture wide variations are seen in the length of the individual bacilli. The ends may be rounded or distinctly pointed, or one may be rounded and the other pointed. The rod is rarely uniform in diameter throughout its entire length, one or more swellings being visible in most individuals. When a single such swelling is present at one end only, the bacillus presents a clubbed appearance, the opposite end being usually pointed : when both ends are enlarged the organism has been loosely compared to a dumb-bell. Other examples may present a single central swelling, the ends being either rounded or pointed. Very short bacilli also occur, often somewhat triangular in shape : two such short forms are often in apposition by their bases, and it is believed that they represent the products of a recent fission.

The wide variations in length, combined with the irregular development of the swellings, give rise to the *polymorphism* with which *C. diphtheriæ* is rightly credited. In order to appreciate the polymorphism it is necessary to study and compare the individual bacilli in a microscopical field : its degree varies with the strain of the bacillus and with the medium upon which it has been grown. Nevertheless, the microscopical picture presented by a film of the diphtheria bacillus is one of considerable constancy in its general effect upon the eye of the observer. The rapid bacteriological diagnosis of diphtheria would indeed be impossible were this not the case.

In stained preparations, especially when weak solutions of dye are employed, the bacillus is seen to take the colour unevenly. Bands of light and dark staining may alternate, or the uneven staining may be quite irregular. The enlarged portions usually stain deeply.

In addition to these irregularly stained areas certain granules can usually be made out, particularly when special stains are employed. These granules, first described by Babes (1886), are not spores, and are now usually called metachromatic, owing to the reddish violet colour assumed by them when stained with methylene blue and certain other dyes. The names polar granules or Neisser's bodies, which are also applied to them, are due respectively to their common position near the ends of the bacilli, and because they can be differentially stained by a process popularized by M. Neisser (1897). The granules vary in number from a single one, situated at one end, or more rarely towards the centre of the organism, to 4 or even 5 distributed along the whole length of the bacillus. Owing to their size they may be the cause of the irregularities in the breadth of the bacillus. These bodies have a great avidity for aniline dyes, and when once stained resist decolorization more than the remainder of the bacillus. Metachromatism is much more conspicuous when the preparation is examined by means of a yellowish artificial light.

When examined by dark-ground illumination, refractive granules can be made out in the body of the bacillus, and, in addition, the contents of the bacillus appear to be barred owing to differences in the refractive indices of its contents. Neither spores nor capsules are ever seen in the unstained preparation, nor can they be demonstrated by staining methods. When specimens of a 20-hour old culture incubated at 37° C. are examined unstained, the bacilli are seen to be non-motile.

The arrangement of the organisms, as seen in a stained film of a culture of *C. diphtheriæ*, is also distinctive. Individual bacilli may lie singly, but much more frequently they occur in small groups of 2, 3, or more members; these may lie parallel to one another or at various angles so as to resemble the letters V or L, or combinations of these which have been compared to Chinese or, better, cuneiform characters. Chains are never seen.

Morphology as seen in Membranes.

When examined in smears from membranes the morphology does not differ from that described above. The organisms are found most abundantly in the more superficial layers, where they usually lie in clumps, being grouped so that they have, not inaptly, been compared to an irregular heap of matches. Sometimes *C. diphtheriæ* may be present to the exclusion of practically all other bacteria, but more commonly the surface of the membrane is occupied by dense masses of cocci and bacteria, among which a fair number of *C. diphtheriæ* may be seen. The deeper layers of the membrane usually contain no bacteria.

Morphology in Cultures.

The bacilli obtained from growths on coagulated serum will be described as the standard forms, since this medium is almost universally employed for diagnostic purposes.

In *general appearance* the bacilli taken from a 20-hour old culture incubated at 37° C. are even more polymorphic than those seen in the false membrane. In length they are found to vary from 1 to 12μ, or even more. Except in the case of very short individuals, perfectly straight organisms are uncommon, the ordinary form being a slightly curved rod, usually with rounded ends, showing, when suitably stained, a metachromatic granule near each end. Bacilli with one or more swellings throughout their length are very common. Clubbed and dumb-bell-shaped bacilli are met with, especially in the longer types. Metachromatic granules are frequently present in the swollen portions, and sometimes in other situations. Occasionally a central swelling is present containing a metachromatic granule, while the ends taper to points.

Other forms seen in larger or smaller numbers are:

Short forms staining almost evenly, usually shaped like two cones with their bases in apposition. These organisms usually have a single bar of light-staining material situated in the middle, which is probably the division line. When metachromatic granules are present they are usually small and are situated in the dark-staining portions.

Such forms are usually considered to be products of recent fission, but occasionally cultures are isolated which consist almost entirely of this type, very closely resembling the pseudo-diphtheria bacillus of Hofmann. This organism is the ' bacille court ' of the French authors, and was first described by L. Martin (1892).

Coccal forms may occur, and Parish (1927) describes an extreme instance of this formation. A culture of the well-known ' Park and Williams No. 8 ' strain on a three days' tryptic digest of horse-muscle at room temperature consisted almost entirely of coccal forms. Recently isolated virulent and avirulent strains cultivated in this manner also developed large numbers of coccal forms. The coccal forms disappeared on the first subculture on Loeffler's serum and on agar. They were virulent and toxigenic, and were not, therefore, degenerative in character.

Streptococcal forms consist of a straight or slightly curved, rather long organism, in the interior of which 4 or 5 somewhat small, regularly placed metachromatic granules may be demonstrated. The organism, when suitably stained, presents a superficial resemblance to a short chain of streptococci surrounded by a capsule.

Sheath bacilli are usually long, curved organisms containing several metachromatic granules of unequal size and irregularly placed. The body of the bacillus usually takes the stain very faintly.

Barred bacilli are organisms of varying length usually without metachromatic granules. When suitably stained they appear as rods composed of alternating bars of light- and dark-staining material.

In any culture practically all these forms may be seen, but the relative number of each form varies in different cultures, and even in those made from the same strain. Indeed, when the different portions of the surface of the same serum slope are examined very great differences may be detected in the proportion of the different types.

Branching forms. In many cultures, but more especially in those consisting largely of the long variety, branching forms can be found when careful examination is made. The usual type consists of a rather long bacillus with a single branch coming off at an angle—approximately a right angle—to the long axis of the parent stem. The branch almost invariably springs from a thickened portion of the bacterial body which usually stains very deeply, but a metachromatic granule is seldom situated at the junction of the branch with the parent stem. The branch apparently commences as a bud on the side of the bacillus, which grows out to varying length and ultimately may break off, forming an ordinary long form. Occasionally, secondary branches may appear on the first branch and from the parent stem other branches may be developed.

L. Martin *et al.* (1924) describe a method by which the ' Park and Williams No. 8 ' strain may be caused to develop branching forms. It is grown in a Martin's broth which has been heated to 70° C. and then sterilized by filtration through a candle. In tubes of this medium covered with oil so as to obtain an anaerobic condition, a film develops at the junction of the broth and oil, which in the course of four or five days shows a profusion of branching forms.

Most of the earlier and a considerable number of present-day observers look upon branching forms as degenerate types or ' involution forms ', but other observers regard branching as a phase in normal development.

Involution forms. When cultures of almost any strain are incubated for more than 24 hours the so-called ' involution forms ' begin to make their appearance, some media being more favourable for their demonstration than others ; for instance, egg medium gives cultures showing a greater number of these forms than Loeffler's coagulated serum.

In cultures which have been incubated for between 24 and 48 hours at 37° C. many of the bacilli will be found to have grown distinctly larger, both length and breadth having increased ; any irregularity in outline will have become more marked. This increase in size and in irregularity of shape continues, so that if specimens are made from cultures 3 or 4 days old many bizarre forms may be seen. The bacilli are faintly stained, but scattered through their substance are large deep-staining masses which may be metachromatic.

Among the common forms may be mentioned :

(i) Large curved rods with a fairly regular outline containing large metachromatic granules at each end, and perhaps one or two others, irregular in size, scattered along the middle portion of the bacillus.

(ii) Club-shaped organisms are common, the enlarged ends being occupied by a large mass of substance taking the metachromatic stain. The body of the bacillus is usually curved and tapers gradually to a point. Other metachromatic granules or dark-staining masses may be distributed irregularly along the body.

(iii) Round or bottle-shaped forms, usually staining deeply, or having a large deep-staining metachromatic mass occupying the major portion of their contents, are also not uncommon.

(iv) Very long forms which contain metachromatic granules more or less regularly placed along the length are also occasionally seen. The last two varieties have been respectively compared to yeasts and portions of mycelium, to which they have a superficial resemblance.

These forms, and others intermediate between them, together with branching individuals, are said to occur more abundantly on medium which is neutral or faintly acid ; but for their demonstration a medium capable of giving good growth of *C. diphtheriæ* must be employed, and not one which inhibits the growth, such as is usually employed for demonstrating the involution forms of other groups of bacteria. Since Loeffler's original publication they have almost universally been described as ' involution forms ', but it is questionable whether this term should be applied to them if it signifies that they are in a degenerating stage.

Several of the more recent authors hold that they are normal to the life-history of the organism, and the same applies to the much rarer branching types. Their constant appearance in cultures grown on media suitable to the growth of C. *diphtheriæ* and their absence from cultures made on media containing a high percentage of sodium chloride, used so successfully for demonstrating the true involution forms of B. *pestis*, give support to this view.

If branching and involution forms are normal stages of the life-history of the organism, C. *diphtheriæ* and the group of bacilli to which it belongs may possibly have a closer relationship with higher fungi than is the case with most other bacteria.

Classification of Strains upon Morphology.

Several attempts have been made to construct a classification of the strains of C. *diphtheriæ* on morphological grounds. The most extensive work along these lines was carried out by Wesbrook, Wilson, McDaniel, and Adair (1898, 1899–1900). These authors, although allowing that every culture consists of a mixture of practically all the forms, yet considered it possible to place the strain in a given group owing to the predominance of some particular type. Different batches of media, and even such differences as the moistness or dryness of the surface of the same medium, may, however, cause variations in the proportions of the morphological types, and it seems practically impossible to lay down any definite classification.

Method of Division.

The reason for the characteristic arrangement of the bacilli in V, L, or cuneiform characters depends upon the mode of division of the bacillus.

The writer observed this by means of hanging-drops of nutrient gelatin inoculated with the organism and kept on a hot stage ; the gelatin melts, but being tenacious, individual bacilli may be watched for a long time with little trouble. A bacillus is seen suddenly to snap at the middle and the two parts assume an L or a V arrangement. It appears that the cell membrane ruptures on one side only and the two parts for a time remain attached by the membrane on the other side. By a repetition of the process among adjacent bacilli, the organisms become pushed together and the parallel grouping is thus assumed. Hill (1898–1902) observed and described this mode of division by means of inoculated slabs of agar stuck on the under-surface of a cover-glass.

STAINING OF C. DIPHTHERIÆ.

C. *diphtheriæ* stains readily with any of the usual basic aniline dyes and also by Gram's method. Methylene blue is one of the best of the simple stains for the purpose and Loeffler's formula (see Vol. IX) can hardly be surpassed for routine work. It shows the beading and segmentation, the involution forms, and the metachromatic granules. Carbol-methylene blue and toluidin blue solutions may also be employed.

As regards the Gram stain, it is generally admitted that C. *diphtheriæ* is Gram-positive, but it does not retain the stain under the action of the decolorizing agent so intensely as many other micro-organisms, nor so markedly as some other members of the group, e.g. the Hofmann bacillus. The original Gram stain (aniline-gentian violet), carbol-gentian violet, Jensen's modification or the method of Claudius (see Vol. IX) all give good results. As counter-stains eosin, neutral red, very dilute carbol-fuchsine, or Bismarck brown may be employed. The organism stains somewhat deeply with the Gram stain, particularly the metachromatic granules, and the picture is not so characteristic as when methylene blue is used.

In order to demonstrate better the characteristic appearances and arrangement of the organism, stained wet preparations have been advocated. A drop of a dilute stain, such as carbol-fuchsine, is placed on a slide, a small portion of a colony of *C. diphtheriæ* is gently emulsified in it, a coverslip is then applied, and the preparation is examined.

Differential staining methods are also much used in the routine examination for the presence of the diphtheria bacillus. Neisser's method, or a modification of it, is probably most widely employed. Neisser's original method (1897) consisted in staining the film (culture or membrane) in an acid methylene blue solution for 2 or 3 seconds, followed after washing by counter-staining in an aqueous solution of Bismarck brown. Later, Neisser (1903) employed an acid methylene-blue-crystal-violet mixture for the first stain, followed by aqueous chrysoidin as the counter-stain. Falières (1902) used a borax methylene blue followed by Bismarck brown; this is stated to be one of the best methods. The writer stains with Neisser's acid methylene blue for 20 to 30 seconds, treats the film with Gram's iodine solution, and counter-stains with eosin. A single stain giving excellent results is an acid solution of toluidin blue (Pugh, 1905). Gutstein (1927) recommends an azur II solution, and McCartney and Harvey (1928) the addition of an azur L dye to acid toluidin blue. By all these methods the metachromatic granules stand out as deeply stained inky dots lying in the lightly stained rods.

Sections of the membrane may be stained with (1) carbol-methylene or toluidin blue and eosin, (2) Gram's method, or Claudius's modification of it, (3) Murray's (1919) stain with Nile blue and van Gieson. (For all these methods, see Vol. IX).

CULTIVATION OF *C. DIPHTHERIÆ*.

General Behaviour of the Bacillus in Artificial Culture.

Media. C. diphtheriæ grows readily on all the ordinary laboratory media, but Loeffler's serum, which is a mixture of serum and glucose broth coagulated by heat, plain coagulated serum, or serum agar are commonly employed, especially for primary cultivation for diagnosis.

Relation to oxygen supply. C. diphtheriæ grows most vigorously when oxygen is abundant. This is especially noticeable in broth cultures when a veil of growth frequently extends over the surface of the medium. In the production of the specific toxin the oxygen is an important factor.

The bacilli, however, are able to multiply under strictly anaerobic conditions. For instance, in deep agar shake cultures the colonies are seen to extend to the deepest parts of the medium, although on or near the surface they are larger and more numerous.

Optimum temperature, &c. On any favourable medium continuous, but very slow, growth takes place even at room temperature (15 to 18° C.), for several weeks. Even at 20 to 22° C. growth is slow. The optimum temperature is 37° C., at which growth is very rapid, but the colonies cease to increase in size after 5 or 6 days. Growth also occurs at 40° C., but above this temperature is said to cease. Klein (1890) stated that in milk *C. diphtheriæ* grows better and more abundantly at 18 to 20° C. than at 37° C., and it grows at room temperature much more rapidly in milk than, for instance, in broth, especially when raw milk is employed.

Emulsions of the bacilli are killed when heated to 58° C. for 10 minutes. Low temperatures, even those of liquid air (—190° C. for 7 days or —252° for 10 hours) do not kill the bacilli.

Colour production. The growth on coagulated serum almost always has a cream or faint yellowish colour. Hill (1903) states that repeated subculture on serum may lead to an increase of colour production, so that a bright yellow may be constantly produced after a few days' growth. The chromogenic substance was soluble in chloroform, but not in water. Occasionally he observed a reddish colour, and the bacterial mass in old broth cultures may have a dirty red brown colour.

Viability of cultures. From cultures on coagulated serum, stored at room temperature and protected from strong light and desiccation, subcultures can usually be obtained after 5 or 6 months, and even longer.

On media containing a considerable percentage of glucose the bacillus usually dies out rapidly owing to the production of acid.

On a medium composed of pieces of meat or blood-clot moistened with tap-water and protected from drying by a layer of liquid paraffin, the bacillus will remain alive for months or possibly even for years.

Characters of Cultures.

Loeffler's medium. Cultures made from the false membrane show, even after 6 to 8 hours' incubation at 37° C., minute colonies of *C. diphtheriæ.* After 18 to 24 hours' incubation the colonies have considerably increased in size, and if not too crowded together may measure as much as 1 mm. in diameter.

As seen by the naked eye the colonies appear as smooth, raised, whitish, translucent, circular spots with a moist shining surface. When abundant and massed together they have a distinct cream or dull yellowish tint, the depth of colour seeming to vary with different samples of serum. When viewed by transmitted light with a lens magnifying 10 to 15 diameters, they are seen to be distinctly granular; the centre, being raised and thickened, appears more opaque than the periphery; the edges are usually quite regular and smooth.

In older cultures the colonies, when separate from one another, increase in diameter so that after 4 or 5 days' incubation a single colony may measure 4 or even 5 mm. in diameter. This growth occurs chiefly at the periphery, and thus the colonies have a more flattened appearance, but the central thickening, although not much increased in size, is more apparent. The surface loses its shining aspect and may become irregular owing to the formation of fissures and ridges, while the edges are indented. When the colonies are crowded together they tend to coalesce into a sheet of growth with a smooth, dull, yellowish surface and a thinner and somewhat irregular edge. This appearance, however, is only observed in primary cultures when the medium is accurately prepared.

When the colonies are magnified about 20 or 25 diameters, the appearance differs according to the age of the culture. After 20 hours' incubation at 37° C., the central portion is seen to be distinctly thickened and heaped up; outside, the peripheral portion gradually slopes to the edge, which is quite sharply defined and regular. The most notable feature, however, is the coarsely granular structure, which gives to the colony a whitish, opaque appearance, a feature which often enables the observer to identify a colony of *C. diphtheriæ* when surrounded by those of other organisms. After 2 or 3 days' incubation the central portion appears even more thickened, and rises sharply from the surrounding peripheral part, which is proportionally larger than in the younger colonies; the edge becomes less regular, and notches and fissures may appear, but these latter usually extend only for a short distance into the colony. Later, folds and ridges may be seen in the peripheral portion. The structure becomes even more granular, and the colony, in consequence, is more easily distinguished from those of other organisms.

In streak cultures a continuous band of growth develops along the track of the needle, broader below than above, yellowish in colour, thicker in the centre than at the edge, which is usually somewhat irregular. The surface in very young cultures is moist and shining, but in the older ones drier and dull.

Ordinary agar. On good nutrient agar, most strains of *C. diphtheriæ* grow well, but not so rapidly as on Loeffler's serum. After 24 hours' incubation the colonies developing on an agar slope are still very small, especially when the culture is made from a false membrane, but after several generations on artificial media growth may become more rapid and abundant.

Two types of colony can usually be seen, a small and a large; the first type is much the more numerous. The small colony is greyish white, somewhat translucent, with a raised, rounded surface and usually a smooth, circular margin; occasionally, however, the latter is irregular and indented. The centre is thickened and, in consequence, appears more opaque, and the structure is distinctly but finely granular. The larger colony is whiter, smoother, very finely granular, and almost always has a smooth, circular outline and a large central thickened portion.

Cowan (1927), by almost daily transplants on serum agar plates for a period of three months, obtained two types of colonies distinct and constant on subculture. She found that although the two types were derived from a virulent and toxigenic stock culture, the 'rough' type was now avirulent and atoxic, the 'smooth' type retaining, however, its virulence. Parker (1928) also observed the same phenomenon by plating on trypsin-serum-agar. On this medium he observed three types of *C. diphtheriæ* which he designated (1) light growers, (2) rough heavy growers, and (3) smooth heavy growers. With many strains, but not all, he found, like Cowan, that the rough strains were atoxic, whereas the smooth strains were toxigenic.

It is questionable if any of these variants exactly correspond to the 'rough' and 'smooth' types of other organisms. The two variants of ancient date do not seem to differ in virulence, neither is similar culturally to Cowan's non-virulent variant, nor has the non-virulent diphtheria bacillus any resemblance in growth to the latter.

In primary cultures on agar, however crowded the colonies may be, they always remain separate from one another, but after repeated cultivation the colonies may coalesce to form a continuous growth.

In stab cultures growth takes place along the whole length of the needle track, appearing as a greyish white line or series of dots. The surface growth consists of a thickened central portion having a smooth, rounded surface with a thinner peripheral border, the margin of which is usually irregular.

In shake cultures growth appears not only on the surface but throughout the whole mass of the agar, showing that the bacillus is a facultative anaerobe.

Streak cultures show a whitish band of growth along the needle track, broadest at the bottom of the tube and tapering towards the top, where it often breaks up into separate colonies. The band of growth is thickest in the centre, and the margins are usually irregular. The structure is finely granular.

The morphology of the bacilli grown on agar differs in several respects from that observed in cultures on serum. In general the bacilli are more regular in shape, metachromatic granules are developed later or not at all; short forms are commoner and the so-called involution forms are not so well developed. The bacilli, however, have the same arrangement, and mostly resemble the intermediate forms of those cultivated on serum.

Glycerin agar. Glycerin agar is superior to ordinary agar as a medium for *C. diphtheriæ*, and agar containing a considerable percentage of glycerin has been recommended by various authors for the isolation of this bacillus. It is, however, now generally accepted that high percentages of glycerin hinder the growth of *C. diphtheriæ*, and that not more than 3 per cent. of glycerin can be added to the agar with advantage. The colonies are similar to those grown on ordinary agar, but they are slightly larger in size. The morphology of the bacilli also resembles that of organisms cultivated on agar.

Glucose agar. Agar containing 1 per cent. of glucose is also superior to ordinary agar as a medium for the diphtheria bacillus ; the growth in all the forms of culture resembles that on ordinary agar, except in the case of the shake culture. When a fairly copious shake culture is made, the colonies develop abundantly in the depths of the medium, but little or no growth occurs on the surface ; in fact, the culture has a superficial resemblance to that of an anaerobic organism. A possible explanation is that the organisms growing in the depths of the glucose agar do not break down the sugar and consequently produce no acid, while those on or near the surface attack the sugar vigorously and produce sufficient acid to arrest development of the colony. In glucose agar stab cultures the line of growth may be thicker in the depth of the medium than nearer the surface.

Other serum agar selective media are referred to under ' Diagnosis ', p. 104.

Gelatin. The cultures on gelatin whether in streak, stab, or shake cultures, are very similar to the corresponding cultures on agar, but the growth in every case is very much slower owing to the lower incubation temperature. The colonies continue to increase in size for several weeks when the culture is kept at room temperature. Even when incubated at 22° C. the development is slow. The gelatin is never liquefied by *C. diphtheriæ.*

When sown on the surface of gelatin and incubated at 20° C., a proportion of the colonies after a varying period increase rapidly in size, and stand out sharply from the others, being both larger and more opaque ; later the increase in size of these colonies ceases and the slower growing ones become larger, until no difference can be detected between the two varieties.

Klein (1890) gives a detailed account of the types of colony seen when the bacillus is grown on the surface of gelatin ; he describes two main types, one of which is the larger, has a granular structure, is greyish white in colour, semi-translucent, with a thickened centre, and usually a smooth, circular outline. This form is by far the more common. In the other type the centre consists of a granular, rounded mass, greyish white and semi-translucent, from which radiate thinner portions evenly separated from one another and resembling the rays of a daisy ; in fact, the whole colony, when magnified, bears a strong resemblance to this flower.

The morphology of the bacilli grown on gelatin shows no distinctive character. Short forms generally predominate, metachromatic granules are not numerous, and the so-called involution forms are often conspicuous by their absence, even in cultures some weeks old. For instance, we have observed that, out of 6 strains grown at 20 to 22° C. for between 1 and 2 weeks, 4 showed only very short forms from 1 to 1·5μ in length, while the other 2 strains showed, amongst a majority of such short forms, a proportion of clubbed, dumb-bell, and bottle-shaped organisms measuring up to 6μ, 7μ and even 10μ in length. The average breadth of these bacilli was about 0·5 to 0·6μ, while the clubs reached 1μ or even 1·5μ. The enlarged ends or swellings were frequently occupied by a mass of material taking the metachromatic stain, and smaller metachromatic granules could be demonstrated in other portions of the organism.

Potato. On potato, especially if its surface has been rendered alkaline, growth *does* take place, although it is often invisible to the naked eye. Occasionally, however, a fine film, smooth, moist and shining, can be made out.

The morphology of the bacilli grown on potato is usually characterized by a number of short, thick, deeply staining forms.

The addition of cystine, 0·0125 gm. per 100 c.cm. of medium, is stated to improve the rapidity and luxuriance of growth on nutrient agar and Loeffler's serum (Braun and Mündel, 1927).

Broth. When *C. diphtheriæ* is planted in ordinary nutrient broth growth is rapid and abundant, and usually has a somewhat characteristic appearance. Although for the first few hours of growth the broth may be evenly clouded, the bulk of it rapidly clears, owing to the bacilli collecting into clumps and sinking to the bottom of the flask; at the same time a film of growth spreads over the surface, its formation commencing at the junction of the surface with the side of the flask. This surface film tends to break up with the slightest movement, and the granules composing it sink to the bottom.

The typical appearance of a culture after 3 to 5 days' incubation is as follows: the bulk of the broth is brilliantly clear, but over the whole surface is a film of growth, which on close inspection is seen to be made up of small rounded clumps. At the junction of the vessel wall and the surface of the broth this film is considerably thickened, and is quite continuous. Sticking to the side of the flask are a variable number of clumps, and the bottom is usually covered with a thick deposit of these rounded masses of bacteria. The slightest agitation causes the whole surface film to break up and sink rapidly to the bottom. The granules of growth, when seen singly, have a greyish white colour, but in mass may have a slight brownish yellow tinge. During the growth of the bacillus the reaction of the broth alters.

The usual sequence of changes is as follows: if the broth is alkaline to commence with, the reaction gradually approaches neutrality, or may in some cases become slightly acid; later still, it again becomes alkaline. The change of reaction is due to organic acids produced in the course of metabolism, and these are later transformed to carbonates producing the alkaline reaction. This is the usual course of events, but if glucose has been added to the broth in amounts of 0·2 per cent. or more, the broth becomes so acid that the growth of the bacilli is inhibited, and, therefore, the secondary alkaline phase is never produced. Abt and Loiseau (1925) state that an appreciable amount of *carbon dioxide* is formed (4 gm. CO_2 per 1,000 c.cm. of broth culture for Martin's broth), part of which remains in solution in the medium, and part is combined to form bicarbonates ; it is derived from amino-acids, particularly glutamic acid (Abt, 1925).

During the growth of the bacillus in broth the toxin is produced, and the question of the reaction has important bearings on its production.

Glucose broth. In broth containing 1 per cent. of glucose, growth is at first very rapid, but the medium soon becomes intensely acid, the bacilli settle down to the bottom and sides of the vessel in an adherent mass, and growth ceases.

Milk. Rapid growth takes place in milk, which becomes acid, but most authors state that no clotting is observed. The preliminary reaction of the milk and the amount of acid produced may be factors which would account for the divergent opinions concerning the growth of the bacillus in this medium.

BIOCHEMICAL REACTIONS.

As already mentioned, *C. diphtheriæ* does not liquefy gelatin or coagulated serum.

Fermentation reactions. The fermentative activity of the Corynebacteria is best determined in Hiss's serum-water medium (see Vol. IX) with litmus or some other indicator and 1 per cent. of the fermentable substance ; change of reaction and in some instances clotting in addition being the indications. Change of reaction occurs within 48 hours, but 7 days should be allowed for the appearance of clot. In this medium, *C. diphtheriæ* ferments glucose, maltose, galactose, glycerin, and dextrin with acid formation, but no gas. Clotting also always occurs with glucose, but some strains fail to clot with maltose, galactose and dextrin. Saccharose, mannitol and dulcitol are never fermented. With regard to lactose, anomalous results have been recorded in the past, but the careful work of Barratt (1924) and of Okell and Baxter (1924), who between them tested 206 virulent, and 131 non-virulent, strains, shows that unaltered lactose is never fermented by *C. diphtheriæ*. If, however, a lactose medium has been sterilized by heat, the lactose may be altered and sometimes may then give an acid reaction with some strains. Barratt further considers that dextrin is a substance of too uncertain composition to constitute a reliable reagent.

Jensen and Falk (1928, p. 372) tested 45 virulent and 5 avirulent (not pseudo-diphtheria) strains of diphtheria bacilli upon 36 fermentable substances in a sugar-free veal infusion broth, with brom-cresol-purple as an indicator. The test substances were dissolved in distilled water, sterilized by filtration, and added aseptically to the prepared tubes of veal broth, so that they were not subjected to the action of heat. Of these 36 test substances glucose, lævulose, galactose, maltose, dextrin and glycerol were fermented alike by all the 50 strains. Acid was not produced from any other of the test substances except glucosamine hydrochloride ; 5 virulent and 1 avirulent culture failed to ferment this substance.

Brown, Duncan and Henry (1924–5) observed that a culture of *C. diphtheriæ* in a broth containing sodium fumarate gives a heavy precipitate with lead acetate; this applies to both the virulent and avirulent strains. *C. pseudodiphthericum* cultured and tested in the same way does not give this heavy precipitate.

Maltase. Sugg, Fleming and Neill (1927) state that a heat-labile, endocellular maltase is present in the diphtheria bacillus, which is liberated when the bacilli are disintegrated.

Bacteriophage. Blair (1924) isolated a lytic principle active for the diphtheria bacillus from the intestinal contents and from the peritoneal exudate of guinea-pigs inoculated with *C. diphtheriæ*, and from diphtheria cultures two months old. The lytic principle was not specific but dissolved not only several strains of the diphtheria bacillus but also members of the typhoid-colon-dysentery group.

Other products. A small amount of ammonia and also organic acids, fixed and volatile, are formed during growth. The latter are derived mostly from inositol and carbohydrate and probably also in part from nitrogenous compounds in the culture medium.

Indole is not formed by the diphtheria bacillus, though cultures give an indole-like reaction with the Salkowski reagent (sulphuric acid and potassium nitrite) after a week or two weeks' incubation (Palmirski and Orlowski, 1895), but not with the Ehrlich reagent. Hewlett (1900, 1901) showed that the substance giving this 'indole' reaction is identical with Salkowski's so-called skatole-carboxylic acid, which was afterwards found by Gentzen to be really indole-acetic acid (Mann, 1906). This finding has been confirmed by Frieber (1921). The indole-acetic acid is derived from tryptophan.

Hæmolysis. Broth cultures of the diphtheria bacillus hæmolyse red blood corpuscles, those of the rabbit being the most sensitive. Filtrates of cultures are without action, which implies that the hæmolytic power is associated with the bacterial cells. The hæmolysis may take from a few hours to four days in the incubator to be apparent, antitoxin is stated to inhibit it, and the reaction seems to be variable with different strains and cultures; the variation may, however, be due to differences in technique. The lysin is thermo-labile and is destroyed in half an hour at 58° C.

Diphtheria Toxin.

The fact that the bacilli remain localized and are not disseminated throughout the body in animals which die after inoculation with diphtheria membrane or culture led Loeffler to infer that at the site of inoculation a poison was produced which on absorption caused the fatal injury. Roux and Yersin were the first to prepare this poison—diphtheria toxin—by filtering well-grown broth cultures of the diphtheria bacillus through a Chamberland filter-candle. Although the filtrate was sterile, its injection into animals caused the development of all the symptoms caused by the living culture, with the exception of false membranes. They also demonstrated that with small doses of toxin, if death did not supervene too quickly, paralysis often occurred (which was not observed by Loeffler), and they made a detailed and accurate study of the conditions under which the toxin is produced.

Toxin production is a very characteristic property of the diphtheria bacillus, and although strains exist which are avirulent and produce no toxin, all strains from the acute sore throat produce toxin in some degree. The amount of toxin formed by different strains varies greatly, and for the preparation of antitoxin, for which maximum toxin-production is essential, only a few strains are available, that known as ' Park-Williams No. 8 ' being generally employed for this purpose. Toxin production may be demonstrated for most strains by growing in broth at 37° C. for some days and then filtering the culture through a filter-candle ; the filtrate on inoculation will be found to be toxic and to produce characteristic symptoms and lesions (see p. 91). For maximum toxin-production a number of details have to be observed, as a medium may permit of abundant growth and yield no toxin. The subject is discussed at some length in the M.R.C's. Monograph *Diphtheria*, pp. 93–116. The composition and mode of preparation of the broth is of supreme importance. Any kind of meat may be employed—ox, veal, horse—though there seems to be a general preference for veal. Some consider the meat should not be too fresh, others stress the use of good fresh meat. The determining factor probably depends in large measure on the content of glucose and muscle sugar, the presence of which in the broth leads to the production of acid, excess of which is detrimental to toxin production. In stale meat the incipient putrefaction tends to remove these substances, and with this object in view preliminary treatment of the meat infusion with *B. coli* has been utilized and is still advocated by Gibbs and Rettger (1927), and for Martin's broth the veal infusion is kept at 37° C. for 24 hours. Povitzky (1929) uses good fresh veal, but even this may fail to yield satisfactory results, and recommends an addition of Liebig's extract to the infusion: — 3 parts of veal infusion + 1 part of Liebig solution (Liebig's extract, 5 gm. ; water, 1,000 c.cm.). Peptone must be present in the finished broth, and the form used exerts considerable influence. Of manufactured peptones, one derived from the digestion of fibrin should be chosen, such as Witte's, the ' Difco ' Proteose of the Digestive Ferments Co., or Park, Davis & Co.'s Bacteriologic Peptone, and 2 per cent. is added. The peptone may also be derived by auto-digestion in the medium, and Douglas's and Hartley's pancreatized meat broths have given excellent results. L. Martin's broth is of this type (see Vol. IX), the peptone being formed by the auto-digestion of pig's stomach ; it constantly yields a high-grade toxin, and is extensively employed. For the preparation of the peptone solution for Martin's broth, Schmidt (1925) states that considerable importance is attached at the Pasteur Institute to the vessel employed, which should be of stoneware or earthenware. Sodium chloride, 0·5 per cent., is commonly added, but this addition is unnecessary for Martin's broth. Over-heating in sterilization is to be avoided so far as possible, particularly if the medium has a reaction on the alkaline side (Hartley & Hartley, 1922; Walbum, 1922; Watson and Langstaff, 1927), a final reaction of pH = 7·3 to 8·0 should be aimed at. During incubation free aeration is essential, and to ensure this the finished broth should form a comparatively shallow layer in the bottles or flasks, e.g. 600 c.cm. or 1,000 c.cm. in Erlenmeyer flasks of 2 litres or 5 litres capacity respectively. With the same object, and to allow carbon dioxide to diffuse freely, the flasks after inoculation are loosely plugged with cotton-wool; they are incubated at 35 to 37° C. for from 7 to 11 days.

The culture for inoculation requires special preparation. The stock culture may be kept on Loeffler's serum at room temperature, with transfers every four days or so. The inoculum should be prepared by inoculating a small flask of the particular broth used with the culture and incubating for 24 hours. A pellicle should form on the broth, and this is transferred with a platinum spiral into a second small flask of broth, and the process is repeated a third time. The toxin flasks are then inoculated from this third culture, the pellicle being transferred. The object is to ensure good pellicle formation which seems to be essential for active toxin formation. Povitzky (1929) recommends first plating the stock culture on veal agar, which yields 'smooth' and 'rough' colonies, and picking out a smooth colony for inoculating the first preliminary broth culture, which should be a 1 per cent. peptone broth if an added peptone is used, the second and third preliminary broth cultures being in the standard 2 per cent. peptone broth. For good toxin production all authors agree that the culture should be acclimatized to the particular broth employed.

For immunization in the preparation of antitoxin the toxin should be free from organisms, and the broth culture after the required period of incubation is therefore filtered through a Berkefeld candle. A 'good' toxin would be one of which 0·005 c.cm. or less of the filtrate of the culture is a fatal dose for a guinea-pig.

The toxicity of toxin is always described in terms of the quantity which will kill a guinea-pig weighing about 250 gm. in 96 hours or thereabouts; this is known as the *minimal lethal dose*, or for brevity the m.l.d.; it can be estimated with considerable accuracy. For this purpose three or four guinea-pigs of 250 gm. weight are first tested with amounts of toxin at somewhat wide intervals, e.g. 0·01, 0·005 and 0·001 c.cm. of toxin respectively. In order to measure these small quantities with accuracy, the toxin is first diluted with sterile saline: for the example given, the preliminary dilution might be 1 in 100. The amounts of this dilution required to give the doses mentioned would be 1·0, 0·5 and 0·1 c.cm. These amounts are accurately measured into small 'test glasses' and the volumes are made up to approximately 4·0 c.cm. by the addition of saline and the whole quantity is injected subcutaneously in the abdomen of the guinea-pig. Supposing the dose of 0·005 c.cm. kills the guinea-pig in about four days, further tests are then performed with doses a little below to somewhat above this amount, e.g. 0·0046, 0·0047, 0·0048, 0·0049, 0·0050 and 0·0051 c.cm., and the smallest of these quantities which causes death within 96 hours is taken as the minimal lethal dose.

It is a remarkable fact that a dose of toxin which does not kill within a little more than 96 hours is rarely fatal under 9 to 10 days. That is to say, a dose ever so little less than the dose which kills in 96 hours does not kill a day or so later as might be expected, but if lethal is not fatal until at least 9 to 10 days have elapsed; swelling with necrosis and ulceration may be present at the site of inoculation, and paralysis may supervene if death be delayed. These effects were attributed by Ehrlich not to the acute lethal constituent, the true toxin, but to another substance ' toxone '.

Diphtheria toxin is unstable and even under the best conditions (addition of toluol and storage in the cold) undergoes progressive diminution in toxicity. This Ehrlich attributed to the conversion of the toxin into other substances, ' toxoids '. Such a toxin of diminished toxicity may still retain its power of combining with antitoxin almost unimpaired, due, according to Ehrlich's views, to the fact that toxoid has lost the toxophore or poisoning groups of toxin, but still retains the haptophore or combining groups which condition the union with antitoxin.

Exposure to light, heating to 60° C., addition of acids and alkalis (see Wallace, 1927), solutions of metallic salts, iodine, and formalin, all cause more or less rapid loss of toxicity of the fluid toxin; a dry toxin, prepared by precipitation or evaporation, is much more stable. Organisms growing in the toxin also tend to destroy it. Inoculating a toxin with certain organisms and incubating at 37° C. for 21 days, Stark, Sherman and Stark (1928) obtained the following results: the incubated but uninoculated toxin (control) contained 175 m.l.d's. per c.cm., inoculated with *B. coli*, 50; *B. cereus*, 25; *P. vulgaris*, 5; *B. pyocyaneus*, 1; *Cl. sporogenes*, less than 0·5, m.l.d.

Toxin may be precipitated, concentrated, and purified to some extent from extraneous matter, by various methods, as by ' salting out ' with ammonium sulphate, alum (see Wallace, 1927), and other salts, or by the addition of 1 to 3 per cent. of acetic acid, collection of the precipitate, and re-solution in caustic soda solution, all the operations being carried out at the freezing-point (see Watson and Wallace, 1924). Abt (1928) adds calcium chloride solution to precipitate phosphates, which are discarded, and then a solution of di-hydrogen-potassium phosphate; di- and tri-calcic phosphates are precipitated and adsorb the toxin, which is obtained by dissolving this precipitate in a solution containing sodium citrate and citric acid, and purifying the solution by dialysis.

The action of iodine and particularly of formalin under certain conditions while destroying toxicity leaves unaffected the antigenic properties of the toxin, which is converted into substances analogous to, or identical with, toxoids ; this antigenic substance is termed ' anatoxin ' by Ramon (1928). He finds that the change into anatoxin is best effected by an addition of 3 to 4 c.cm. of formalin (40 per cent. formaldehyde) to 1,000 c.cm. of toxin and keeping the mixture at 38 to 40° C. for one month. Less formalin is required if the reaction of the toxin is pH = 7 than if it is pH = 8, and an amount of formalin sufficient to effect the transformation at 39° C. is not enough at 37° C.

Diphtheria toxin, derived from whatever strain, appears always to be the same substance, in so far as it is capable of being neutralized by one and the same antitoxin. It is true that Havens (1920) asserted that the toxins produced by his two serological races are not identical, and are unequally neutralized by stock antitoxin, but a mass of evidence conflicts with his results, which are not generally accepted. Okell and Parish (1926) found that a monovalent antitoxin, derived from the Park-Williams No. 8 strain neutralized all of 819 virulent strains.

The chemical nature of diphtheria toxin is unknown ; the active principle forms but a very small fraction of the toxin broth (Watson and Langstaff, 1926). Warden, *et al.* (1921) claimed that the toxic agent is a fatty substance, and that real diphtheria toxin can be produced by colloidal emulsions of ordinary fatty acids and broth under particular conditions ; the latter part of their statement cannot be confirmed (Dernby, 1926). Locke and Main (1928) state that purified preparations of toxin contain no carbohydrate and are lipoprotein in character. The mode of formation of the toxin is not clear. It may be a normal excretory product of the bacillus, but the fact that growth and toxin formation are not necessarily parallel does not support this hypothesis ; excellent growth is not always followed by potent toxin production. Or it might be an abnormal product of bacterial metabolism, and only formed provided the organisms have certain substances available. Several facts support this view, notably the influence of the composition of the culture medium and of apparently minor factors upon toxin production. Another hypothesis suggests that the toxin may be set free by autolysis of the bacilli, and Walbum (1922[1]) has shown that toxin may be produced by triturating washed diphtheria bacilli and allowing them to stand in contact with nutrient broth at 37° C., and postulates the existence of a ' protoxin ' in the organisms. Dernby and Walbum (1923) suggest that the greater part of the toxin is derived neither intracellularly nor from autolysed bacteria, but originates in the following manner. The diphtheria bacilli grow, die, and undergo autolysis, thereby liberating specific proteolytic enzymes, which split the proteoses and peptones of the medium and of the bacilli with the formation of lower degradation products. Some of these intermediary split-products constitute the toxin, and inasmuch as the major portion of the proteoses is present in the culture medium, on this hypothesis the greater part of the toxin has an exogenous origin. This hypothesis would explain the importance both of the composition of the culture medium and of the strain of diphtheria bacillus on toxin production, and the diminution of the toxin content by prolonged incubation might be explained by a continuation of the proteolytic degradation process with further splitting of the toxin and loss thereby of its specific properties (see Dernby, 1926). Locke and Main (1928) also agree that the toxin is not a disintegration or extraction product of dead bacilli (see also Fujioka, 1928; Gallinek, 1928).

Endotoxins. The name 'endotoxin' has been given to the toxic principle contained within the bodies of the diphtheria bacillus, and there is experimental evidence that the washed bodies of the bacilli and certain extracts thereof exert a poisonous action distinct from that of the toxin proper. Injection of the toxin-free bodies produces necrosis and suppuration at the site of injection, and in sufficient doses they are capable of killing guinea-pigs in from 6 to 10 days with cachexia, local lesions and parenchymatous degeneration of organs. The endotoxin is distinct from the toxin, for it may be heated to 100° C. without destruction, toxin being rapidly destroyed at 60° C., and ordinary antitoxin does not neutralize it. With endotoxin prepared by mechanically grinding the washed and frozen organisms, Hewlett (1912) was able to confer on guinea-pigs considerable protection against inoculation with living diphtheria culture. Accumulated experience, however, has led to the general belief that endotoxin does not play any significant role in the causation of the symptoms of diphtheria.

Serological Reactions.

Antitoxin is the most important of the serum antibodies derived from the diphtheria bacillus. Some amount, admixed with other antibodies, may be obtained by the inoculation of an animal with cultures of *C. diphtheriæ*, but by far the greatest yield is obtained by the treatment of an animal with increasing doses of toxin (or of anatoxin; for the preparation, properties, and standardization of antitoxin, see Glenny, Vol. VI). The horse is by far the best animal for antitoxin production: the goat may be used, but is less satisfactory: the rabbit is a poor antitoxin producer and the rat does not react at all. Antitoxic serum, as prepared by the injection of strong toxin derived from young cultures, usually contains little or no agglutinin, bacteriolysin, and complement-fixing bodies. It does contain substances which exert a stimulating action on the phagocytes *in vivo* and *in vitro*. This action has been demonstrated both with the heated serum, which, therefore, contains thermo-stable opsonins or bacteriotropins of Neufeld, and with the fresh serum, which is therefore opsonic in Wright's sense.

Anti-endotoxin. By injecting animals first with dead and then with living bacilli, previously washed free of toxin, sera have been prepared which will neutralize the endotoxin and so prevent its ill effects, even when the endotoxin is injected in the form of living bacilli.

Agglutinins. Agglutinating sera may be obtained by inoculating the rabbit or horse (not the guinea-pig) with emulsions of serum cultures of *C. diphtheriæ*. Bell (1922) experienced a good deal of trouble, with a heavy death-rate among the rabbits. The method finally adopted was to inject the animals intravenously first with emulsion killed by heat, followed 5 days later by a dose of living culture. The dose of living culture was repeated at 5-day intervals for a period of 3 months. The dose during the first month was 500 million organisms, during the second month 1,000 million organisms, and during the third month 2,000 million organisms. A small dose of antitoxin (0·1 c.cm.) was also given with every dose of culture. At the end of the treatment the average agglutinating titre of the sera was 1 in 400. For the tests, emulsions of the organism killed by heat were mixed with equal volumes of the serum dilutions, the mixtures were placed in a water bath at 55° C., and read after 4 hours. The emulsions contained 3,000 million organisms per c.cm. Eagleton and Baxter (1923) used emulsions of virulent organisms killed by heat and containing 2,000 million organisms per c.cm. The rabbits were inoculated intravenously with 1, 2 and 3 c.cm. of this antigen on consecutive days ; no antitoxin was given. After an interval of 8 or 10 days, an experimental bleeding was taken ; 3 c.cm. of antigen were then injected, and after another interval a second test was made. Nearly half the animals now had a titre of 1 in 400 or more, and if so their serum was used. Eagleton and Baxter consider that agglutinating sera deteriorate rapidly even when stored in the cold. Bell examined 133 strains of the diphtheria bacillus and found that 80 per cent. of them belonged to three types. Eagleton and Baxter examined 348 virulent strains, and by direct agglutination were able to classify in 10 groups 323 strains—including 13 which gave agglutination with the sera of groups 3 and 4—leaving 25 strains unclassified. By means of agglutinin-absorption tests, a further 9 of the unclassified strains absorbed agglutinin from one or other of the type sera, finally leaving 16 strains unclassified. They found that positive cultures from the nose and throat of the same patient taken at the same time, and strains cultured from the same patient at different times, almost always showed the same serological type of organism. It was also found that sera could be prepared from avirulent strains with which virulent strains could be classified, and vice versa, but the serological reactions of the avirulent organisms were much less sharply defined than those of the virulent ones. It is thus seen that many serological races of *C. diphtheriæ* exist, and the value of the serological investigation of the organism appears to be epidemiological rather than bacteriological, for, broadly speaking, in a particular epidemic one serological type prevails.

Agglutinin-absorption and *complement-fixation tests* may be carried out with the sera prepared for agglutination, but except for defining more closely some strains that agglutinate with two type sera, or do not agglutinate, do not yield information of practical value. Stone (1923) did not find complement fixation as serviceable as agglutination for differentiation of serological groups of the diphtheria bacillus.

Bacteriolysin does not appear to be present in either antitoxic or antimicrobic diphtheria serum.

Precipitin. Antitoxic serum mixed with toxin (or anatoxin) yields a flocculent precipitate. The reaction under standard conditions is a quantitative one, and is now employed as one of the methods for the standardization of antitoxin (see Glenny, Vol. VI).

Pathogenic Action of C. Diphtheriæ and Pathology of Diphtheria.

Experimental diphtheria in animals has been the subject of much research, and Loeffler and other early investigators showed that *C. diphtheriæ* is pathogenic for a number of animals, prominent among which is the guinea-pig. Experimental inoculation of living cultures or of toxin free from organisms yields very similar results, and accumulated experience has led to the general belief that the symptoms of diphtheria are caused by intoxication with the toxin, the endotoxin of the bacillary bodies playing but an insignificant role, if any, both in experimental and in natural diphtheria. It will be convenient to consider first experimental diphtheria in animals in order of their importance, and finally natural diphtheria in man.

EXPERIMENTAL DIPHTHERIA IN ANIMALS.

Guinea-pigs.

Routes of inoculation.

The mere application of cultures of *C. diphtheriæ* to the intact skin of mucous membranes of guinea-pigs is rarely if ever capable of inducing experimental diphtheria. A lesion of some kind appears to be essential. The most commonly employed method is *subcutaneous* or *intramuscular* inoculation. After *intraperitoneal* inoculation the evolution of the experimental disease is relatively slow. Inoculation of the *intact vulva* is successful in 25 to 50 per cent. of the animals, the most characteristic lesion being local necrosis with swelling of the regional lymph nodes and œdema of the uterus. Culture applied to the *intact cornea* usually produces little effect, but if the cornea be pricked with a needle dipped in culture varying degrees of keratitis ensue. Diphtheria bacilli injected into the *mouth, nose* or *trachea*, or inhaled in a spray, generally produce no effect unless a preliminary lesion of the mucous membrane has been induced, when a small proportion of the animals may show membrane formation and become infected. Introduced into the *stomach* they are similarly without action. *Intracerebral* injection of diphtheria toxin is more deadly than when the toxin is introduced subcutaneously, and minute doses which are not immediately lethal may be followed by late paralysis.

In general, it is not possible to carry on a series of passages from an animal inoculated with living culture to other animals, apparently because the organisms rapidly diminish in numbers after introduction into the body. A second animal inoculated with membrane or œdema fluid from the first one dies only after considerable delay, and the third animal inoculated from the second usually survives.

General symptoms.

When an acutely lethal dose of diphtheria culture or of diphtheria toxin is injected subcutaneoulsy into a guinea-pig of 200 to 400 gm. there is no immediate result. After a latent or incubation period, which after large doses may be reduced to about 12 hours, a soft, doughy, œdematous swelling forms at the site of inoculation and may extend for a considerable distance in all directions. By the end of 24 hours after inoculation the animal usually manifests symptoms of serious illness. It will be found cowering in a corner of its cage, with coat ruffled and with definite dyspnœa. It is cold to the touch. In young growing animals there is at first perhaps no loss of weight, but even an actual increase, which, however, soon ceases, and the weight diminishes rapidly up to the moment of death in about 4 days (Fig. I). A dose of diphtheria culture or toxin which will kill in this time is spoken of as the 'minimum lethal dose' (m.l.d.). Multiples of this quantity will kill guinea-pigs in as short a time as 24 hours with symptoms correspondingly violent and acute.

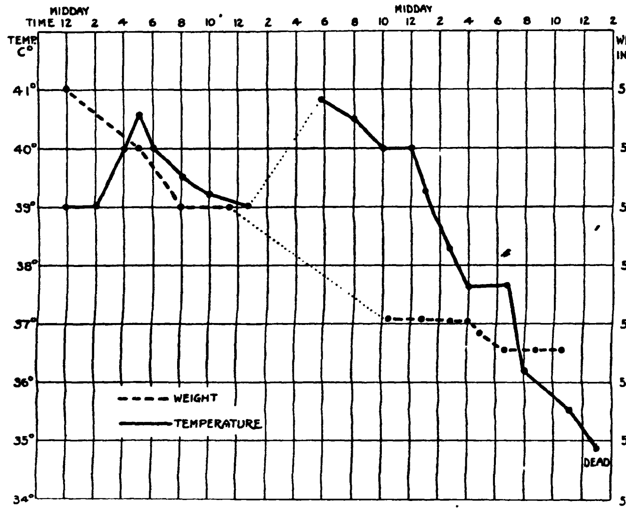

FIG. I. The effect of diphtheria toxin upon the guinea-pig (Madsen, 1896).

By means of calorimeter experiments it has been shown that there is a true incubation period followed by a stage of increasing hyperthermia, which then decreases, to be followed by marked hypothermia in the last stages of the illness, the loss of temperature being secondary to the gradual failure of the vital powers of the animal (Fig. I).

When the culture is not in its most virulent state or the dose is small, the animal may survive for a week, or even much longer, and finally succumb in a state of marasmus. The local lesion, at first soft, becomes indurated ; necrosis sets in and the whole affected area may slough and leave a slowly healing granulating wound.

General lesions found post mortem.

At the autopsy of a guinea-pig dead from acute diphtheria intoxication after subcutaneous inoculation of culture or toxin, the most striking lesion is the massive, trembling, gelatinous and sanguinolent œdema at the site of the inoculation, extending through the thickness of the abdominal walls to the serosa. Enlargement and congestion of the lymphatic glands in the axillæ and groins are also prominent. The vessels of the abdomen generally are distended and engorged with blood, and an exudate, clear or cloudy and sanguinolent, and often in very considerable quantity, will be found in the pleural cavities and to a less extent in the peritoneum and pericardium. The abdominal viscera are congested and a striking condition is seen in the case of the adrenal glands, which are enlarged and of a colour which varies from reddish yellow to dark red. When cut into they are commonly found to be hæmorrhagic. According to Pritchett (1922[1]) the hæmorrhage begins at about 36 hours and increases up to 48 to 72 hours, after which it recedes. It is usually most marked in animals that have received large doses of toxin. When death ensues rapidly (18 to 24 hours) or is delayed up to or beyond 4 days, Pritchett found that it might be slight or absent. The lungs are often œdematous and several authors have noted necrotic areas in the liver. With regard to the incidence of these several lesions, J. Homer Wright (1894), in a careful analysis of 160 autopsies, found a manifest local lesion in 145 (90 per cent.), enlargement and congestion of lymph glands in 142 (88·7 per cent.), pleural exudate in 68 (42·5 per cent.), œdema of the lungs in 82 (51·2 per cent.), congestion of suprarenals in 130 (81·2 per cent.), and necrotic areas in the liver in 49 (30·6 per cent.).

In a series of 50 experiments with virulent diphtheria cultures, Barratt (unpublished) used 2 c.cm. of a well-grown 48-hour broth culture and found the mortality and the incidence of the lesions as follows:

		Cases.	*Percentage.*
Time of death	24 hours	7	14·0
	48 ,,	36	72·0
	72 ,,	7	14·0
Lesion at site of inoculation	Injection of vessels only	3	6·0
	Slight œdema	12	24·0
	Moderate œdema	18	36·0
	Extensive ,,	17	34·0
Pleural cavity	No fluid	28	56·0
	Small quantity of fluid	7	14·0
	Moderate ,, ,,	12	24·0
	Large ,, ,,	3	6·0
Adrenal glands	Pink	2	4·0
	Red	11	22·0
	Deep red	37	74·0

From this it is manifest that the congestion of the adrenals is the most constant lesion of experimental diphtheria.

In the more chronic forms of the experimental disease the above lesions may all be absent and the animal presents chiefly the signs of marasmus with or without the appearance of a healing wound or scar at the site of the inoculation.

Distribution of the bacilli in the body.

The early investigators found that in death from the injection of diphtheria cultures, diphtheria bacilli were not disseminated throughout the body; indeed, it was this fact that led to the conception of diphtheria as essentially an intoxication process. They never found diphtheria bacilli in the heart-blood in experimental diphtheria although they were occasionally seen microscopically in, and frequently recovered by culture from, the local lesion. These findings are, however, true only when the inoculum is relatively small. When death follows massive doses diphtheria bacilli can be found in the pleural exudate, the heart-blood, lymph glands and viscera.

Detailed consideration of individual lesions.

Local exudate or infiltration. In guinea-pigs dying very acutely, say in 48 hours, the local lesion is found to be of the nature of an inflammatory œdema in which there are many leucocytes showing by their staining properties the evidences of degeneration. No definite fibrinous network can be made out, the exudate being amorphous. Tissue into which the exudate has been poured will be found necrotic, and that which immediately underlies the epithelium is, in addition, heavily infiltrated with leucocytes. The exudation of fluid may extend through the muscles underlying the site of the inoculation, in the case of the abdominal wall—the usual site of the inoculation—to the peritoneal cavity. The vessels of the muscles are mostly congested and exhibit many leucocytes in their lumina. In some cases actual thrombosis may have taken place. These changes occur both with toxin and with living cultures, although they are more marked in the latter case.

In guinea-pigs surviving up to four days or more the local changes will be found to have altered, in that marked necrosis has set in, the skin and underlying tissues being heavily involved. Extensive collections of leucocytes will be found in foci, which, if the inoculum has been large, may contain many bacilli engulfed by a phagocytic process. With small doses of culture, phagocytosis is not evident. After complete necrosis has ensued the dead part separates, leaving an open granulating wound which cicatrizes in the course of time.

Ledingham (1926) describes the histology of the local lesion in guinea-pigs dying 24 hours after subcutaneous inoculation of culture. There is a diffuse infiltration with polymorphonuclear leucocytes extending to the epidermis, which shows pyknosis of the nuclei of the Malpighian cells. The vessels are greatly congested and there are occasional hæmorrhages with much œdema in the part. The reticulo-endothelial system of the area, including the capillary endothelium, the adventitial cells of the sheath and the histiocytes along the connective tissue fibres, all show evidence of karyorrhexis and pyknosis, terminating in transformation of their nuclei into large deeply staining spherical or ovoid masses. There is no delimitation of the leucotaxis and only a small amount of phagocytosis of the bacilli by the leucocytes.

The regional *lymphatic glands* are enlarged, and after the inoculation of culture congestion, hæmorrhages, and swelling of the reticulum and vascular endothelium are found ; with toxin alone the changes are mainly of a degenerative character. A number of deeply staining bodies, very variable in size, are found throughout the sections ; these are probably degenerate nuclei.

Effusion into the pleural cavity is common and the quantity of fluid may be 10 c.cm. or more ; it is sometimes slight or absent. The fluid may contain a large proportion of mononuclear and eosinophile leucocytes, particularly when toxin has been injected. Some strains of *C. diphtheriæ* appear to have a greater capacity to produce pleural exudation than others. Œdema of the *lungs* is common and congestion of the vessels marked.

Changes in the *spleen* and *liver* are as a rule not marked. Congestion, parenchymatous degeneration, and necrotic foci may be present and nuclear fragments may be abundant in the spleen. The *kidneys* in diphtheria toxæmia or infection are usually congested and albuminuria is frequent. The epithelium of the convoluted tubules shows cloudy swelling and degeneration, and Pritchett (1922[1]) has directed attention to a peculiar lesion of Bowman's capsule, consisting of proliferation and swelling of the capsule cells, which may assume the appearance of cubical epithelium.

Adrenal glands. The characteristic lesion of experimental diphtheria, which is so frequently met with in the adrenal glands, has been the subject of much investigation, though the exact nature of the changes is not yet completely understood. Extravasations of blood are found in the cortex and medulla, the hæmorrhage being most marked in the medulla. Most observers describe the lesions of experimental diphtheria infection or intoxication as being most marked in the cortex, consisting of congestion and hæmorrhages, degeneration of the cells, and marked diminution of the chromaffinic elements. The content of adrenaline is probably much reduced as a rule.

The walls of the *alimentary canal* show in general congestion, hæmorrhages, and an œdematous condition of the mucous membrane, and ulceration of the stomach is met with in more than half the animals.

Heart. Parenchymatous changes first occur and are directly caused by the action of the diphtheria toxin, followed later by interstitial changes which are inflammatory and secondary. In acute toxæmia the earliest changes consist of congestion of the vessels and œdema of the muscle fibres. Swelling of the endothelium follows with infiltration of round cells and polynuclear leucocytes around the vessels. In animals dying on the third or fourth day definite extravasations of blood are found under the epicardium and between the muscle bundles, and interstitial changes with collections of leucocytic and other cells become evident. Parenchymatous alterations of a fatty character commence early and become more and more marked if life is prolonged. If death is delayed for a week or two, the interstitial changes become prominent. Newly formed cells are arranged in rows along the muscle bundles, and collections of round cells and leucocytes are found in the neighbourhood of vessels and under the endothelium; later, proliferation occurs with the formation of granulation tissue.

Direct injection of the myocardium with toxin causes extensive degenerative and inflammatory changes—cloudy swelling, fatty change, and necrosis—and infiltration with leucocytes (Apfelbach, 1925).

Nervous system. Guinea-pigs rarely suffer from paralysis as a result of the injection of toxin alone, but with toxin-antitoxin mixtures when the toxin is not quite neutralized paralysis is not uncommon if life be prolonged beyond 8 or 10 days. This effect was attributed by Ehrlich to the presence in the toxin broth of epitoxoid (toxone). The paralysis may be general, affecting chiefly the muscles of the neck, back, abdomen and hind legs, and local paralysis may occur in a limb near the site of injection. Histologically, the brain and spinal cord are hardly affected, but most of the peripheral nerves show typical Wallerian degeneration of the myelin sheaths, the axis cylinders remaining intact.

Rabbits.

Weight for weight, the rabbit has a susceptibility about the same as that of the guinea-pig to inoculation of diphtheria culture and of toxin. The general description which has been given of the symptoms, lesions, &c., in the guinea-pig apply also for the most part to those in the rabbit. False membrane, which may be of considerable extent, is readily produced by intraconjunctival or intratracheal inoculation, particularly after a slight lesion, and the intratracheal injection of culture after tracheotomy is followed by symptoms reminiscent of ' croup ' with dyspnœa. The liver is the site of extensive fatty degeneration, and acute glomerular and tubular changes are present in the kidneys. The adrenals are very red and congested.

Rats and Mice.

Rats and mice possess a high degree of resistance to diphtheria culture or toxin. The fatal dose of toxin for the rat by subcutaneous injection has been estimated to be 3,000 to 4,000 times, and for the mouse to be 60 to 100 times, the subcutaneous m.l.d. for the guinea-pig. These animals also exhibit a high degree of tolerance to living culture, but massive doses may be fatal. Kolle and Schlossberger (1920) state that $\frac{1}{2}$ to $\frac{1}{50}$ of a loop of a fresh serum culture is regularly fatal to white mice in 3 to 8 days. The immunity of these animals is not humoral, for their serum has little neutralizing power for toxin *in vitro*, and must be considered to be cellular or histogenic, or possibly their cells are less permeable to, or have less power of fixing, toxin than those of the guinea-pig.

Ledingham (1926) ascribes the immunity of the rat to the reticulo-endothelial system, which by rapid proliferation delimits the leucocytic area and ultimately replaces the leucocytic zone and forms a barrier to generalization ; there is no pyknosis (contrast with the guinea-pig, p. 92). There is marked phagocytosis of the bacilli by the polymorphonuclears, and to some extent also by the mononuclears. The reticulo-endothelial system of the rat is indifferent to the diphtheria bacillus and its toxin, the bacillus acting merely as an innocuous foreign body.

Dogs.

The dog is susceptible to all methods of inoculation of culture or of toxin, and weight for weight is only slightly more resistant than the guinea-pig. The general symptoms, lesions, &c., are similar to those met with in the guinea-pig and rabbit. In acute experimental diphtheria bilious vomiting, diarrhœa with passage of blood, jaundice, and intense albuminuria occur, and if life be prolonged for 8 or 10 days paralysis may follow.

Cats.

E. Klein (1889, 1890, 1891), as a result of a number of experiments, concluded that cats are susceptible to inoculation with living culture and even to feeding, and he ascribed to cats a possible role in the transmission of human diphtheria. Klein's results have not subsequently been confirmed and are not accepted to-day. He seems to have based his deductions of the pathogenicity of the diphtheria bacillus for the cat mainly on an appearance of fatty degeneration of the kidney ; actually a pale and fatty condition of the kidney cortex is normal in the cat, the epithelium of the cortical tubules being loaded with fat globules. More recently, Savage (1919–20) has reinvestigated the subject of experimental diphtheria in kittens 4 to 6 weeks old. Inoculation with 2 c.cm. of a 5-day old broth culture was followed by death in the course of a few days, but large quantities of culture swabbed on to the throat, even after the application of silver nitrate, or injected into the nose, produced no effect, and ingestion of vast quantities of virulent diphtheria bacilli was similarly without result. The diphtheria bacilli not only failed to infect, but failed to survive in the nose and throat more than a day or two. Animals inoculated subcutaneously may become paralytic, and after death the adrenals show the usual changes.

Monkeys.

The monkey seems to be comparatively insusceptible to diphtheritic infection. In Burnet's (1910) experiments on nine chimpanzees the application of culture or of human diphtheritic membrane to the throat and nose, even after the mucous membrane had been injured, was generally almost without effect. Cultures of *C. diphtheriæ* never produced lesions, such minor effect, as exudation, that occurred resulting from the application of membrane.

Other Animals.

Our knowledge of experimental diphtheria in the *cow, sheep, goat* and *horse* is meagre. Subcutaneous injection of culture in all these animals causes local swelling and œdema and may be followed by death. Sublethal doses of toxin in the sheep, goat and horse cause a sharp reaction, with local infiltration, fever and anorexia. Ledingham (1907) observed paralysis in the goat following a large dose of toxin, and Madsen (1896) describes paralysis in a horse which had received three doses of toxin.

Birds seem to be susceptible to inoculation of culture and of toxin. Frogs inoculated with toxin are unaffected if kept at room temperature, but at 38° C. show signs of paralysis.

EXPERIMENTAL AND ACCIDENTAL INFECTION IN MAN.

Many intentional or accidental inoculations of mucous membranes, e.g. throat and conjunctiva, and of skin incisions in man with fragments of diphtheritic membrane and with culture have been recorded. A preponderance of negative results have followed these inoculations, presumably because these individuals possessed an immunity. This presumption is probably correct because most of the inoculations were done in adults, who are generally much less susceptible than children, but is incapable of proof, as the existence of immunity to diphtheria was not determined in any of these cases prior to inoculation. To elucidate this question, Guthrie, Marshall and Moss (1921) inoculated 8 healthy volunteers, of whom 4 gave a strongly positive Schick reaction, and 4 a negative. Emulsions of virulent bacilli were rubbed over the throat, with the result that the carrier state was established in 7 out of the 8 individuals, the exception being a person who had previously had a dose of antitoxin. The individuals with a negative Schick reaction escaped infection, but the four with a positive reaction developed typical diphtheria, one with considerable membrane formation being very ill. All recovered with antitoxin.

NATURAL DIPHTHERIA IN MAN.

It is not easy to give an account of the lesions in man produced by *C. diphtheriæ* alone, because of the frequency of secondary infections which blur the picture.

The Local Lesions.

Locally, the inflammatory exudate which gives the disease its specific pathological character occurs mostly on the tonsils, palate, uvula, larynx, or trachea, and appears in the form of spots or patches of a greyish white colour, becoming yellowish later on. Between the patches the mucous membrane can be seen to be intensely congested. At first the patches are thin, but they rapidly thicken to a so-called false membrane, and are easily detached or not according to the site where they occur, depending apparently to a large extent on the variety of epithelium involved. Where this epithelium is of a stratified squamous character, the false membrane offers considerable resistance to forcible removal, whereas in mucous membranes covered with ciliated columnar epithelium the attachment is much less tenacious, the false membrane being easily detached, or, indeed, spontaneously expelled from the body in the form of a partial or complete cast of the affected part. If the membrane persists for some time it becomes of dark yellowish brown or almost black colour, and may be associated with a foul odour.

In the main, the membrane is a fibrinous exudate which at first makes its appearance between the epithelial cells, which, later, succumb and may be recognized as homogeneous masses in early specimens. The majority of observers hold that the fibrin owes its origin to plasma which has exuded from the blood-vessels, and has subsequently coagulated.

It would appear that the diphtherial exudate first occurs in the horny layers of the epithelium, and is at first of a viscid rather than of a fibrinous character. Probably the deep epithelial cells contribute to its formation. Even from its earliest production, the exudate is not, however, composed of mucus, as it is capable of coagulation. Apparently the initial damage done by the diphtheria bacillus is to the cells, and is of a degenerative nature ending in necrosis ; to this is early superadded an exudation of plasma from the underlying vessels. This plasma clots and is the main source of the fibrin subsequently found.

Regional lymphatic glands. The lymphatic glands corresponding to the seat of membrane formation are often enlarged, congested, and œdematous, and the cut surface may show yellowish foci. The glandular lesion may be regarded essentially as an extension of the local process, and Bulloch and Schmorl (1894) found a fibrinous exudate in the form of bands and network in the sinuses and follicles of the regional glands. In the follicles they found necrotic foci and in very severe cases the glands were actually hæmorrhagic. From the fact, however, that similar lesions may occur in glands in which no diphtheria bacilli can be found, Bulloch and Schmorl formed the opinion that the changes were due to the diphtheria toxin which was absorbed in relatively concentrated form from the primary lesion in the vicinity.

Lungs. The lungs are frequently involved by continuity from the infected upper air passages, and the changes may thus be regarded as still representing the local effects of the diphtheria virus. The pulmonary lesions are mostly of a broncho-pneumonic character with serous, cellular, fibrinous, or hæmorrhagic exudate. Broncho-pneumonia was found in 60 per cent. of Mallory's (1908) series of 251 cases. Interstitial changes may accompany the parenchymatous and exudative ones, and are often associated with secondary atelectasis and emphysema.

General Effects.

In addition to its local effects, the diphtheria bacillus is capable of producing general lesions mainly through the activity of its toxin, which is absorbed into the circulation. It is generally agreed that the brunt of the mischief is borne by the heart and the nervous system, although other organs, notably the kidneys, adrenals, and liver, may be seriously damaged. The gross anatomical lesions met with after death are very varied, and it is not always possible to separate the action of the diphtheria bacillus from that of other micro-organisms which may have escaped from the local lesion into the circulation.

Heart. Symptoms of cardiac injury are very frequent in diphtheria; they occur early or late in the course of the disease, and may lead to sudden death. Although various lesions have been found and alleged to be the cause of heart failure in diphtheria, the true pathology cannot be regarded as completely known. In a large number of cases lesions occur both in the parenchyma and in the interstitial tissue, and they are particularly well defined in cases which have survived some considerable time or in the graver clinical forms of the disease.

Fatty infiltration has been frequently observed either in the form of fine or of coarse droplets, and the lesion may involve extensive areas. One of the latest investigations is by Warthin (1924). He describes the essential lesion of the myocardium as consisting of a toxic parenchymatous hyaline degeneration, frequently associated with fatty infiltration, and less frequently with cloudy swelling or a simple necrosis. If the patient survives, a reparative inflammatory process (myocarditis) follows with regeneration of the muscle, and either complete regeneration may result or fibrosis may ensue. The bundle of His is attacked much in the same way and to the same extent as the myocardium, that is there is elective action on the bundle.

A local eosinophilia is described as occurring in diphtheritic myocarditis, which is not found in other infective diseases.

The cardiac failure which is so common in diphtheria has naturally been attributed to the cardiac lesions which are frequently so evident. But cardiac deaths occur in which only slight lesions are found in the myocardium. Romberg (1891) therefore suggested that the circulatory disturbances of diphtheria are caused by paralysis of the vasomotor centre and collection of much of the blood in the splanchnic area. A great deal of clinical and experimental work resulted from this suggestion of Romberg, but the general trend of opinion has returned to the heart as the main part involved in the circulatory failure.

Others have invoked changes and disorders of function of the special conductive apparatus of the heart to explain cardiac collapse. But an examination of the sino-auricular node and bundle of His by many observers has failed to establish any constant lesion, and in many cases these structures are unaffected.

Adrenal glands. Marked naked-eye lesions are but rarely found in the adrenal glands of persons dead from diphtheria. At the same time there is an accumulation of evidence that these organs may be severely affected, and their function, disordered thereby, may account for some of the effects seen in diphtheria. Microscopically, round cell infiltrations, hæmorrhages and thromboses have been described, but the chromaffin tissues appear to be unaffected, and degenerative changes are not marked except in proximity to hæmorrhages.

Kidneys. The action of uncomplicated diphtheria upon the kidneys is not uniform. All parts of the kidney may be involved, although the parenchymatous changes are more frequent and more severe than the interstitial. In the convoluted tubules the epithelium shows cloudy swelling, fatty degeneration or complete necrosis, the lumen being filled with blood or with casts of a homogeneous, hyaline, or actually fibrinous nature. In the straight tubules near their openings hæmorrhages are common. The Bowman's capsules and glomeruli sometimes take part in the process, and the space between the two may be filled with an amorphous exudate or with actual blood. Interstitial and vascular changes, when they occur, are most marked in the cortex.

Spleen. The spleen is not characteristically altered in diphtheria if we except the foci of large cells which Bizzozero (1876) and Waschkewitsch (1900) found almost constantly in children dying of the disease. Several fine histological alterations, such as stasis with hæmorrhages, hyperplasia of the cells, and characteristic phagocytes in necrotic foci, have been described.

Liver. Cloudy swelling of the cells is often present and areas of necrosis around the hepatic veins in the lobules are very common. Round cell infiltrations and small or large focal necroses, with or without an inflammatory reaction, are frequent in Glisson's capsule.

Nervous system. Some degree of paralysis is common in diphtheria, being present in about 20 per cent. of all cases. The paralysis affects, in order of frequency, the palate, accommodation, limbs, trunk and neck, larynx and external ocular and other muscles. It is caused by a peripheral neuritis involving, wholly or in part, the nerves associated with the affected part. The lesion is a parenchymatous one, the fibres are swollen, and the medullary sheath is filled with fine or coarse globules. The axis cylinder may persist for a time, but is liable to become involved later. Interstitial changes are unusual if we except the nerves of the palate. Alterations have been described in the ganglion cells of the anterior cornua of the spinal cord and in Clarke's column, but they are probably exceptional, and, if they occur, the ganglion cells cannot as a rule be seriously damaged, as complete recovery generally ensues.

The frequency with which the palate is involved in faucial diphtheria—the usual site of the primary infection—and the rarity of palatal palsy in extrafaucial diphtheria, have led to the view that the process is an ascending neuritis. The fact that extrafaucial diphtheria may in rare instances be followed by paralysis of the palate, and the frequent involvement of the ciliary and other muscles, have suggested another hypothesis, viz. that the toxin carried by the blood exercises an elective affinity for the nerves of these regions.

The muscles of paralysed regions are often intact, but in long-standing cases may show a considerable collection of fatty granules, a change which has been interpreted as degenerative. This may occur particularly in the diaphragm and intercostal muscles, and is probably secondary to a nerve lesion rather than due to the direct action of the toxin.

The Distribution of C. diphtheriæ *in Natural Diphtheria.*

Although the classical feature of diphtheria is an intoxication due to a microbe which does not itself penetrate into the tissues of the body, there appears to be no doubt that in many cases some degree of dissemination does take place. Blood cultures have been made upon numbers of cases of diphtheria during life but *C. diphtheriæ* has been isolated only very rarely. *Post mortem,* the organism has been isolated more frequently, particularly from the lungs, also from the blood and abdominal organs, and exceptionally from the brain and cerebrospinal fluid. It may be said that while general dissemination of the organism does take place, the number of bacilli in the blood and organs is small and perhaps transient, and this factor is probably not of much importance in the pathology of the disease.

Local Forms of Diphtheria.

Several forms of localized diphtheritic infection are described, but they are of rare occurrence. One of the commonest is a nasal infection, usually termed ' fibrinous rhinitis '. Whether membrane is present or not, virulent diphtheria bacilli can be isolated. An outstanding and anomalous feature of most of these conditions is their extreme mildness ; they are rarely accompanied with obvious constitutional disturbance or complications.

True diphtheritic *conjunctivitis* is rare, and with membrane formation appears to be unknown.

Diphtheria bacilli have been reported to have been isolated from the discharge in *otitis media,* which is alleged to occur as a complication of diphtheria. In most of the instances, the organism isolated was not submitted to full bacteriological tests, and non-virulent diphtheroids are not infrequent in such discharge.

Diphtheria of the *genital organs* is of rare occurrence. Van Saun (1923) describes two cases of diphtheritic *vaginitis* in children, and commenting on the rarity of this condition states that she has been able to find only 26 cases recorded in the literature of the last 30 years.

Cutaneous diphtheria is of more common occurrence than some of the foregoing conditions. It may take the form of ulcers more or less covered with membrane, or of pustular or herpetic lesions. Virulent diphtheria bacilli can be isolated and antitoxin is curative.

Infections of *wounds* with *C. diphtheriæ* occasionally occur.

Laboratory Diagnosis in Diphtheria.

It must be recognized in the first place that the diagnosis of the *disease*—diphtheria—is the province of the clinician alone, and that the responsibility for the diagnosis cannot be placed upon the bacteriologist.

All that the latter can do is to affirm the presence or absence of the diphtheria bacillus in the material submitted to him for examination. His findings may be of the utmost value to the clinician in making up his mind, but it is only one factor in the diagnosis. The presence of the bacillus does not prove the case to be one of diphtheria, nor does failure to find it on the part of the bacteriologist exclude that disease. If the clinical aspect of the case warrants a diagnosis of diphtheria, the clinician will rightly persevere in the use of antitoxin treatment in face of a negative laboratory report ; on the other hand, he may elect to regard the case as one of scarlet fever, even though the diphtheria bacillus is present. With such decisions the bacteriologist has nothing whatever to do. But if the clinician is to derive the greatest advantage from the laboratory report, he should take care that the bacteriologist is afforded full information as to the nature and source of the material submitted.

The clinician has become so convinced of the value of the laboratory diagnosis in diphtheria, that there is a tendency to rely too much upon it and to withhold antitoxin until the bacteriological report is received. *The administration of antitoxin should never be delayed in any case that is clinically manifest diphtheria, nor indeed in any case in which the disease is suspected.* The only justification for awaiting a laboratory report is when the presumption is on the whole *against* diphtheria.

A complete laboratory diagnosis is also impossible in 24 hours. In that period all that can be done is to examine films of the material provided and of primary cultures made from it, and the bacteriologist makes his diagnosis on the morphological appearances of the organisms present. Fortunately, in the acute sore throat, in which an early diagnosis is most important, Corynebacteria other than *C. diphtheriæ* and *C. hofmanni* are exceptional, the characters of these two organisms are generally sufficiently well defined to enable them to be distinguished in the films that have been prepared, and non-virulent forms of *C. diphtheriæ* are rarely present in these circumstances.

It is another matter, however, when the material is derived from convalescent cases or suspected carriers, or from the nose, eye, ear or skin. In the convalescent and carrier non-virulent forms of the diphtheria bacillus are frequent, and in the other regions mentioned Corynebacteria other than *C. diphtheriæ*, and scarcely distinguishable from it on morphological grounds alone, are of common occurrence. The liability to error is greater still in the recognition of the diphtheria bacillus in material derived from animals, milk, or any source apart from the human body.

It follows, therefore, that whereas a *presumptive diagnosis* of the diphtheria bacillus on morphological grounds alone may be justifiable in acute sore throat and laryngitis, far more caution is required in other cases. Caiger and O'Brien (1924) closely studied 436 suspected diphtheria cases in order to gain some information how far the clinical might agree with the laboratory diagnosis. The two agreed in 97·5 per cent. Examination of the first swab gave a correct result in 95·5 per cent., and in only 1·15 per cent. was a negative from the first swab changed into a positive by examination of a second swab. The cases were fairly representative of both positives and negatives—59 per cent. of the former and 41 per cent. of the latter.

THE COLLECTION OF MATERIAL FOR EXAMINATION, AND ITS TRANSMISSION TO THE LABORATORY.

The best material for examination is a portion of membrane removed from the affected region with sterile forceps: the operator can see what he is doing, and exercise selection in the choice of a suitable fragment. Alternatively, a platinum loop can be employed to remove faucial secretion, and this method has the advantage that the end of the loop can be bent at a right angle so as to reach the tonsillar crypts or other recesses. These procedures, however, imply that the bacteriologist has access to the patient and can make his cultures on the spot.

In practice, the material must usually be secured by a person unskilled in bacteriology and various methods have been used for the purpose. Nothing better has been devised than a cotton-wool swab on a stiff wire, sterilized and enclosed in a narrow test-tube of stout glass, which was introduced by v. Esmarch in 1895. The wire should be stiff and long enough to reach to the bottom of the culture tube to be inoculated. It may be looped at one end, for convenience in holding, and notches should be made with a file at the lower end, for the secure attachment of the cotton-wool. The wool employed should be non-absorbent and it should be twisted into a smooth uniform pad, not too large. When the swab has been fitted into a clean test-tube plugged with cotton-wool at the mouth, or provided with a cork, the whole should be sterilized by dry heat or in an autoclave.

When prepared swabs are not available, they may be extemporized in various ways. Cotton-wool may be wrapped round the end of a piece of wire, a straightened hair-pin, a knitting needle, a stick of wood or a penholder, and failing cotton-wool the swab may be formed of a strip of lint or gauze (not antiseptic), or of calico, or a bit of sponge, or a wrapping of darning cotton, &c. The swab may be sterilized in boiling water, or by singeing in a flame, and for transmission if no test-tube is available it may be enclosed in a bottle which has been rinsed with boiling water. Discharge may even be smeared on a piece of paper, or a piece of membrane may be wrapped in paper, the paper in each case being sterilized by flaming.

In swabbing the throat it is important to have a good view of the fauces, and a spatula is usually necessary. The swab should be seen to come into actual contact with the faucial exudate and should be used with such gentle force as to remove some of it. That more failures do not occur from neglect of these precautions is due to the fact that, even when the affection is limited to a part of one tonsil, the diphtheria bacilli can be found in other parts of the mouth. In swabbing contacts there is naturally no exudate to remove: all that can be done is to rub the swab thoroughly over the fauces; and this is true also of laryngeal diphtheria. In obtaining material from the nose the swab should be so introduced as to reach the turbinal bones, as these are the usual seat of membrane: it is difficult to control the procedure by actual inspection. Material from the eye and ear and from wounds is easy to secure. In no case should any antiseptic have been recently applied to the affected area.

Immediate cultivation doubtless offers the best prospect of success, but the diphtheria bacillus in the exudate offers considerable resistance to drying and it is possible to obtain almost equally good results from swabs and other material taken 24 hours previously. In sending material through the post it is necessary to comply with the postal regulations.

IMMEDIATE PRESUMPTIVE DIAGNOSIS OF THE DIPHTHERIA BACILLUS.

Two methods are available for the presumptive diagnosis within 24 hours. The first is direct microscopic examination of stained films of the secretion, the second is cultivation on a suitable medium. Of these methods the second is by far the more certain and must invariably be carried out, but there are reasons why, as a preliminary measure, the first should not be neglected. These reasons are three in number. (1) It sometimes happens that the diphtheria bacillus is present in such large numbers and in such typical form that a diagnosis can be made with almost as great certainty as from a culture. If the case is one in which the earliest possible provisional report is desired, there is a manifest saving of time when such a report can be offered from the direct film. (2) In rare cases of true diphtheria, cultures may be negative although the bacillus can be seen in the direct film. (3) If direct staining be omitted, cases of Vincent's angina—a condition which may simulate diphtheria—will be overlooked, since the ordinary cultural method does not reveal the characteristic organism of that affection.

On the other hand, the cases in which the direct film gives a positive result are, for the most part, cases of clinically manifest diphtheria in which the saving of time is of little consequence, and this method has not, on the whole, found favour with British bacteriologists; nevertheless, it cannot be doubted that it may sometimes be of value.

Provisional Diagnosis of the Diphtheria Bacillus from the Direct Film.

This method should be strictly limited to cases of acute faucial diphtheria, and even in these a provisionally positive report should never be given unless the bacillus is present in numbers and is characteristic in its morphology. *A negative result has no value.*

The proportion of cases in which the bacillus can be found by direct examination depends in some measure upon the experience of the observer, upon the staining method employed, and upon the freshness of the material; under the best conditions it probably amounts to 25 to 30 per cent.

For the direct examination films are prepared; this procedure with exudate or membrane presents no difficulty. In the case of a swab the primary considerati*o*n *is cultivation. It is often possible to find on the* swab a little adherent exudate which can be removed with forceps or with a platinum loop and used for making the films. Failing this, films must be prepared from the swab *after* it has been used to inoculate the culture medium. If the material has become dry, it must first be softened by moistening with sterile saline.

The staining methods employed will be those on which the worker is accustomed to rely, but among them Neisser's stain or one of its modifications is usually considered desirable.

Presumptive Diagnosis by Cultural Methods.

Culture media sloped in test-tubes are made use of in most laboratories, though a few may employ plates as a routine.

Nutrient agar is useless, as the extraneous organisms grow profusely upon it, and overgrow any diphtheria bacilli that may be present. A selective medium is therefore necessary, and for this purpose plain inspissated serum yields perfectly satisfactory results, though Loeffler's modification of it is somewhat better. Other media have been recommended but the only one that needs notice is one containing a tellurium compound, such as the serum-calcium-tellurate of Conradi and Troch (1912), or the trypsinized serum-agar with potassium tellurite of Douglas (1922). Allison and Ayling (1929) recommend as a differential culture medium for diphtheria diagnostic work a trypsinized serum tellurite copper sulphate agar, consisting essentially of Douglas's medium with an addition of 0·05 per cent. of copper sulphate (see Vol. IX). It is claimed that the growth of staphylococci and streptococci on this medium is prevented and the spread of *B. proteus* inhibited, while *C. diphtheriæ* and other diphtheroids grow unrestrictedly and the differential appearance of their colonies is accentuated.

In inoculating the culture tube, the swab (or other material) should be rubbed thoroughly over the surface of the medium, care being taken not to break up the surface. The swab, if dry, should be moistened with sterile saline or in the condensation water in the tube so as thoroughly to moisten it, and the serum tube should not be so old as to be at all dry. The inoculated tubes are incubated at 37° C. as a rule, though it is stated that the Neisser staining reaction succeeds better when the incubation temperature is 35° C.

The diphtheria bacillus grows so vigorously on serum at body temperature that its colonies are the earliest to appear. It follows that the earlier a culture is examined after the bacilli have had time to develop their proper characters, the less will the result be obscured by the growth of other organisms. A positive culture examined after an incubation of between 5 and 6 hours at 37° C. will probably show the bacillus, even though no colonies are apparent to the naked eye, and it will show little else. But at this stage of growth, although the bacilli are large and well formed, *with barred or segmented protoplasm, the metachromatic granules have not yet developed. These commence their development* after about 6 hours of incubation, and in impression preparations, made from serum plates after 6 hours and stained with acetic acid toluidin blue, minute granules are visible towards the poles of many, but not all, of the bacilli. At the end of 8 hours they are well formed and conspicuous, and it is a common experience that at this time a provisional diagnosis can be made. Thus, if swabs are received early in the day a report may often be given by the evening. More usually it happens that the cultures are incubated overnight and examined next morning.

Cultures which prove negative in 24 hours should be further incubated and re-examined on the second day. This is perhaps not a routine practice, but unquestionably some cultures are found positive on the second day which on the first day were apparently negative.

Examination of cultures. Though at times it may be possible to recognize and pick off an isolated colony of the diphtheria bacillus this is usually not feasible, and it is generally preferable to take a sweeping of the mixed colonies on the surface of the culture, particularly from the edge or from the upper part of the mixed growth. The material thus removed from the culture is then examined microscopically, either in a wet preparation, unstained or stained, or in a dry stained film ; the choice must be determined by the custom of the individual worker. Practically any method to which the observer has become accustomed is the ideal one for him, for any change of method involves the construction of a new picture of the characters of the organism.

For wet staining Pugh's acid toluidin blue yields excellent results, but most workers employ dried films. For staining these, Loeffler's methylene blue probably gives as characteristic a picture as any stain to those accustomed to its use. The banded staining is well shown and the metachromatic granules are sufficiently distinct. Neisser's stain, however, renders the granules more sharply visible and many bacteriologists give it the preference, or Neisser's stain may be reserved as a confirmatory method for those specimens in which the Loeffler's blue staining is less characteristic than usual, or in which the diphtheria bacilli are scanty. Pugh's acid toluidin blue is another stain which gives admirable results. As a routine, Gram's stain is not to be recommended.

DIAGNOSTIC CHARACTERS OF *C. DIPHTHERIÆ*.

There is usually little difficulty in determining the presence or absence of bacilli conforming to the classical picture of *C. diphtheriæ :* doubt arises only *when the bacilli depart in some respects from this picture.* The polymorphism of the bacillus has already been stressed (see p. 71), and the forms which it assumes have been described.

The commonest form encountered in diphtheria diagnosis is the long and slender type, tending to be thicker at one end than the other, but in the young culture without pronounced clubbing. With care, the mere shape of the organism usually suffices to distinguish it from bacilli belonging to other genera, but certain streptococci common in the throat often assume in the young serum culture a bacillary form with irregular staining, which may mimic the diphtheria bacillus and must be distinguished from it ; Neisser's stain at once dispels the illusion.

The short type of *C. diphtheriæ*, less commonly met with, presents greater difficulty in diagnosis. Though not so stout as *C. hofmanni*, it may be confused with that organism where a simple stain is alone employed. The chief difficulties, in any case, arise in distinguishing between *C. diphtheriæ* and other corynebacteria. Such difficulties occur even in throat cultures : in cultures from the nose and elsewhere they are formidable.

The metachromatic granules, in a well-stained film, are of the greatest help in discriminating between *C. diphtheriæ* and *C. hofmanni*. In a serum culture between 8 and 24 hours old, the former shows them in marked form, the latter practically never, or so feebly developed and in so few individuals, that mistake is scarcely possible, though even apart from the granules the morphology of the two organisms is distinctive to the experienced eye. It has already been pointed out that these are the two species of Corynebacterium which chiefly occur in the throat, and this is the reason why Neisser's stain is of such great value in the morphological diagnosis of diphtheria. When the question arises of distinguishing between the diphtheria bacillus and corynebacteria other than Hofmann's bacillus, this stain loses a great part of its differential value, for many other diphtheroids, of no pathological importance, may show metachromatic granules as well developed as in *C. diphtheriæ*, or even better, though they do not appear so early. Thus we return to the proposition already laid down, that a presumptive diagnosis is likely to be correct in a case of sore throat, while in cultures from the nose, eye or ear the margin of error is so much greater that the cautious worker will never pronounce a positive opinion.

Even in throat cultures the possibility exists that bacilli conforming in every morphological respect with the diphtheria bacillus may prove on testing to be non-virulent. In acute sore-throat this is seldom the case, though carriers of the non-virulent bacillus may suffer from simple tonsillitis. But in the case of contacts and carriers, the bacilli found prove so frequently to be non-virulent (and, as it seems, harmless), that virulence tests should be carried out before any serious administrative steps are taken.

Before leaving the subject of metachromatic granules as revealed by Neisser's stain, it may be well to point out that these structures are not confined to the genus Corynebacterium. Other bacilli, such as the hay bacillus, show them, though less conspicuously, with this stain ; and the various Gram-negative cocci which are often abundant in the throat show large and deeply staining granules, and two cocci at the right distance apart may have a superficial resemblance to a diphtheria bacillus unless care is exercised. A motile Gram-negative organism, staining by Neisser's method like *C. diphtheriæ*, and growing into long threads on serum, is not infrequent, and has been stated to be derived, not from the throat, but from the cotton-wool of imperfectly sterilized swabs.

Now and then the observer may be in doubt about the nature of the organism present—while conforming on the whole to the characters of the diphtheria bacillus, to the experienced eye it may not be typical. In such cases, the culture should be further examined in a wet specimen for motility, and by Gram's method. The grouping of the organism should be taken into account—the individual bacilli in a group of *C. diphtheriæ* never quite touch one another. It is sometimes worth while to inoculate a second serum tube from a favourable spot on the primary culture ; examination of this may yield less equivocal results.

The virulence of the primary culture is sometimes tested without resorting to isolation of the bacillus. This may be done to save the time required for isolation, but it must be seldom that the urgency is such as to require this ; it may be useful occasionally when failure attends the attempt to isolate in pure culture. For this crude test, the culture may be emulsified and injected subcutaneously into a guinea-pig. But with this method, the death of the animal may be caused by pyogenic cocci or other organisms present in the mixed culture, and a better method is an intracutaneous test first devised by Havens and Powell (1922) and further tested by Force and Beattie (1922) and Bull and McKee (1923). The growth on the original diagnostic culture is washed off with 1·0 c.cm. of saline. This emulsion is diluted with more saline if diphtheria bacilli are numerous, the object being to obtain an emulsion containing about 100 million bacilli in 1·0 c.cm. Two large guinea-pigs, 400 gm. in weight, are employed, of which one has been given 250 units of antitoxin on the previous day; they suffice for testing 4 to 6 cultures. The skin being shaved or the hair removed with a barium sulphide depilator, each animal is inoculated intracutaneously with 0·1 c.cm. of the culture emulsion, and the tests are read after 72 hours. The criterion of virulence is superficial necrosis of the skin, absent in the antitoxin control: mere discoloration or œdema counts for nothing. Okell and Parish (1926) have tested this procedure and find it reliable, provided the examination of a stained smear of the culture shows that not less than one-third of the organisms present resemble *C. diphtheriæ*.

Importance was at one time attached to the association of the diphtheria bacillus with other organisms, some of which may be responsible for local and secondary complications of the disease. The organisms which have been held responsible for such complications are *Staphylococcus pyogenes*, the *Pneumococcus*, and hæmolytic *Streptococci*. The presence or absence of these organisms is best determined by the use of blood-agar plates. When a serum culture from a membranous sore throat fails to yield the diphtheria bacillus, a blood-agar plate may reveal the presence of some other organism as the possible causal agent, though caution must be exercised in excluding *C. diphtheriæ*. Organisms other than the diphtheria bacillus which have been credited with the power of membrane formation on rare occasions in the throat are the *Pneumococcus*, *Streptococcus* and Friedländer's *Pneumobacillus*. Toxic agents, such as scalding water and steam, acids and alkalis, &c., must also be borne in mind in this connection.

THE COMPLETE DIAGNOSIS OF THE DIPHTHERIA BACILLUS.

In conditions other than the acute sore throat, in which the probability of error is small, the nature of the presumptive *C. diphtheriæ* present should be verified by other tests. This applies to swabs from the nose, ear, eye or other regions, or from contacts or suspected carriers. The process of verification consists in isolating the suspected bacillus in pure culture, and then submitting it to certain routine tests, including that of virulence.

For isolation, serum is an unfavourable medium, and it is common experience that an apparently pure colony of *C. diphtheriæ* on serum yields a mixed growth when subcultured on to agar. No culture of *C. diphtheriæ* should, therefore, be deemed pure until it has passed the test of growth on an agar slope. In order to isolate, single colonies, if they exist, may be picked off and streaked on to an agar plate or on to agar slopes, or if the colonies are too crowded to allow of this, a trace of growth from a likely spot is diluted and plated out in the same manner on agar, though in this event Douglas's tellurium-serum-agar, or Allison and Ayling's copper sulphate modification of it (see above, p. 104), presents distinct advantages.

A pure culture having been obtained, the organism is then submitted to certain routine tests, which comprise the following :

Staining tests. These are needful to ensure that a morphologically correct organism has been selected. A simple stain such as Loeffler's methylene blue is as good as any. Granule stains, such as Neisser's, are less suitable for cultures on agar than for serum cultures, so that if it is desired to employ these it is best to subcultivate again on serum.

Fermentation tests. Only three sugars need be employed in routine testing, namely, glucose, galactose and saccharose. A 1 per cent. solution of these sugars in peptone water or Hiss's serum water, with an indicator such as litmus, or Andrade's more delicate bleached acid fuchsin, will be found to give clear results, often apparent in 24 hours, though 4 days should be allowed to elapse before the final reading. There should be evidence of good growth in the tubes pronounced to be negative. The diphtheria bacillus is quite constant in its reactions : it produces acid in glucose and galactose, never in saccharose. Hofmann's bacillus is equally constant in producing no acid in any of the three sugars. The use of saccharose enables us to dismiss the saccharose fermenters. If saccharose is not fermented, but glucose and galactose yield acid, the bacillus may be

C. diphtheriæ in its virulent or non-virulent form : sugar tests do not allow us to distinguish between them. If glucose alone of the three sugars is fermented the possibility of *C. diphtheriæ* may be dismissed.

The virulence test. This is the only certain and satisfactory confirmatory test for the diphtheria bacillus, for no other species of the genus Corynebacterium occurring in man produces a fatal effect upon the guinea-pig. Moreover, it possesses the advantage of discriminating between the pathogenic organism and its non-virulent ally, which no other test will do. Animal experiment is thus a necessary part of the complete diagnosis of *C. diphtheriæ,* and is of fundamental importance in the recognition of carriers.

Either of two methods may be employed. In the first one, the whole of a serum slope is emulsified and injected subcutaneously into a guinea-pig, the death or survival of the animal being the test of virulence ; to render it more complete, a second animal may be similarly injected with culture and antitoxin in addition. The unprotected animal should die within 4 days with the presence of the lesions already described. The second method is the intradermal one described below.

As a subsidiary test, the capacity to grow anaerobically may be determined by means of a glucose-agar shake-culture incubated for 48 hours. *C. diphtheriæ* develops throughout the whole depth of the medium, whereas the colonies of most other diphtheroids develop only at or near the surface.

Serological methods for the recognition of the diphtheria bacillus must at present be regarded as being of little value.

Intradermal Tests.

Intradermal or intracutaneous methods of testing both for virulence of the bacillus and for the presence of minute amounts of antitoxin in serum were first worked out by Römer (1909). He found that an amount of diphtheria toxin corresponding to 1/500 to 1/250 of the subcutaneous m.l.d. produced a local lesion when the material was injected strictly intracutaneously in a dose of 0·1 c.cm. With the minimal doses a slight redness and swelling appear lasting 3 or 4 days, and followed by local loss of hair; a medium dose causes a small weal ending in necrosis, and a large dose (1/50 m.l.d.) is followed by definite necrosis. The method was afterwards extended to the testing of virulence of cultures and to estimating the amount of antitoxin in the blood when it is present in small quantities.

The *intracutaneous method for testing virulence* is now generally carried out by a technique devised by Eagleton and Baxter (1921, 1922) and described in detail by Okell and Parish (1926). The organism to be tested is first isolated in pure culture by plating the original culture on Loeffler's medium in Petri dishes. The colony of *C. diphtheriæ* is picked from the plate preferably on the third or fourth day of incubation when it is most characteristic, though if the bacilli are numerous, colonies can generally be picked off at an earlier stage, e.g. 48 hours. If an organism should be present which liquefies the Loeffler medium, it may be necessary to resort to plain nutrient agar or tellurite agar plates. At least two colonies are selected and sown on Loeffler slopes. After incubation for about 18 hours, smears are made and if found pure the growths are emulsified in saline and standardized by an opacity test so as to contain about 500 million organisms per c.cm. Two guinea-pigs of 250 to 350 gm. weight are required for each series of tests, preferably light in colour or with white patches. One of the animals, which serves as the control, is injected intraperitoneally with 500 units of diphtheria antitoxin the night before the test. The guinea-pigs are shaved or depilated with a barium sulphide depilator. Both the unprotected and the control guinea-pigs are injected intracutaneously with 0·2 c.cm. of each of the standardized suspensions ; a dozen injections, i.e. 2 colonies from 6 cultures, may easily be put up on each guinea-pig. About 6 hours after the injections a 'following' dose of 125 units of diphtheria antitoxin is given intraperitoneally to the unprotected or test animal ; otherwise it might die from diphtheria toxæmia before proper readings are made. It is as well if several suspensions are injected to use two test animals, instead of one, to guard against vitiation of the tests owing to the death of one of them. The animals should be examined daily after inoculation, though as a rule the readings are best made on the second or third day. The lesion on the test animal produced by a virulent culture consists of a small erythematous patch about 15 mm. in diameter which progresses to superficial necrosis within 48 to 72 hours, becoming a dry scab a day or two later. Avirulent organisms produce little or no reaction. On the control (protected) guinea-pig there is practically no reaction or, at the most, a raised red papule which rapidly subsides on the second or third day. 'Pseudo' reactions rarely occur and can generally be distinguished from the true ones. It is advisable to include a control inoculation with a known virulent strain of *C. diphtheriæ*. The various intradermal inoculations should be not less than half an inch apart.

Under the name of '*electrophoresis*', Jensen and Falk, and Falk, Jensen, *et al.* (1928) suggest a physical method for determination of virulence which possibly has a future before it. A shallow rectangular cell is employed with a platinum electrode fixed at either end. The cell is filled with a thrice-washed suspension in distilled water of the culture to be examined, and a large cover glass is applied. The preparation is placed on the stage of a microscope, a level is focussed in the suspension in the cell, current is turned on and the organisms are seen to travel towards one or other electrode. By means of a micrometer eyepiece and a stop-watch, the rate at which the bacilli travel towards the electrode can be determined. After several measurements have been made, the current is reversed and a fresh set of determinations are made. The observations are averaged, and the velocity is calculated in microns per second.

The dividing line between virulent and avirulent strains was found to be a rate of about 8 microns per second. Virulent bacilli were found to travel slower than this, at 2 to 6 microns per second, avirulent ones quicker, at 8 to 20 microns per second. Various Hofmann strains were also tested and travelled still slower. A comparison of the method with animal tests gave a correspondence of 94·4 per cent. in detecting typical *C. diphtheriæ*. For details of the method, the original papers must be consulted.

The Serum Treatment of Diphtheria.

All cases clinically diagnosed as either definitely or probably diphtheria should be given antitoxin at once without waiting for the result of the bacteriological examination, which should always be carried out. It is probably advisable to give a dose of antitoxin even in those cases in which the disturbance of health is extremely slight.

The strength of diphtheria antitoxin is always stated in ' units ', and for practical purposes the unit may be defined as the amount of the antitoxic serum which will just neutralize 100 m.l.d. of diphtheria toxin for the guinea-pig (see Glenny, Vol. VI). The dosage is described in terms of the number of units administered, and every phial of antitoxin has the number of units contained in 1·0 c.cm. marked upon it. The antitoxin is usually the serum as obtained from the immunized horse, but when the dosage is required to be large, concentrated antitoxins can be obtained.

MODE OF ADMINISTRATION.

Although subcutaneous injection is frequently used for the therapeutic application of antitoxin, as it is the easiest, and commonly supposed, though probably erroneously, to be safer than other methods; it is in all other respects inferior to intravenous or intramuscular injection. The absorption of antitoxin into the blood is much slower from the subcutaneous tissue than from muscles. Moreover, with the subcutaneous method a smaller proportion of the injected antitoxin reaches the blood than is the case with the intramuscular (or intravenous) method. When injected intravenously, there is, of course, no delay in the access of the whole dose of antitoxin to the blood-stream, and the maximum concentration is therefore reached at once. But this is not so when the intramuscular or subcutaneous route is adopted. In the former case some 24 hours, and in the latter about 3 days, are required for the appearance of the maximum concentration in the blood.

The object in antitoxin-treatment is to attain an effective concentration of antitoxin in the blood with the least possible delay, and the ideal method, therefore, would always be the intravenous, for all cases of whatever degree of severity. Next to that comes intramuscular injection, and last of all the commonly practised subcutaneous method. These facts leave no justification for continuing the practice of subcutaneous injections in any type of diphtheria.

Considering that the intramuscular route allows of the rapid access of very considerable amounts of antitoxin to the blood-stream, and that the attainment of the greatest concentration is not very greatly delayed, it may be considered admissible to make use of it in mild cases and in early cases of moderate severity.

In severe or late cases the intravenous route ought to be adopted, either alone, or in conjunction with intramuscular injection. If intravenous injections prove to be so safe that the whole of the antitoxin required by a patient may be injected at once into the veins, as Park (1921) recommends, this will effect a considerable saving in the cost of treatment, since the alternative routes of injection require a larger dose to achieve—more slowly—the same effect.

Some modern authorities recommend the administration, in severe cases, of a large initial dose of antitoxin, part intravenously (up to 20 c.cm. of serum) and the remainder intramuscularly (Bie, 1922).

In the intravenous method, the injection should be slow—10 to 20 c.cm. in 20 minutes—with careful watching of the patient and immediate cessation should signs of reaction appear. In dealing with previously injected persons, 'desensitization' by the preliminary injection of small doses of serum may be practised, but with slow injection seems to be unnecessary.

There is little or no evidence that antitoxin can be absorbed from the stomach or rectum. The older observations on this subject have been confirmed by Grasset (1926).

Inasmuch as diphtheria endotoxin possesses a toxic property distinct from that of the toxin, and antitoxic serum does not protect from endotoxin, it has been suggested that sera possessing antibacterial, in addition to antitoxic, power would have a greater therapeutic efficiency than simple antitoxin, but this proposition remains at present unproved. It has also been surmised that antitoxic power is not the only significant property of therapeutic serum, other characters, e.g. 'avidity' for toxin, being believed by some to account for variations in curative power independent of content of antitoxin, and this view has received some support clinically. By 'avidity' is meant the rapidity with which combination of toxin with antitoxin is completed in a toxin-antitoxin mixture. This factor varies with different antitoxin and has been stressed by Madsen and Schmidt (1926) (see Glenny, Vol. VI). The orthodox view is, however, that a serum depends for its preventive and curative power primarily upon its antitoxic strength.

DOSAGE.

Good results were obtained in the early days with moderate quantities of somewhat low-titre sera, but there has been a tendency towards a steady increase in the dose (of antitoxin-units), and in this country it has been the custom for years to give several thousand units in mild cases, and doses of 6,000 to 10,000, or even up to 50,000 or 100,000 units, in very severe infections. The fact is that it is easy to give too little, but it is very difficult to give too much.

According to Thomson (1921), if given on the first day of the disease, the dose should range from 6,000 units for the mild cases, up to 30,000 units for the very severe cases. If given after the first day of onset, these doses should be increased somewhat; on the fourth day or later by 30 to 50 per cent. MacIntyre (1926) considers that the following scale of doses should be regarded as a minimal one—5,000 units for mild cases, 15,000 units for moderately severe, and 32,000 units for severe cases, but 70,000 to 100,000 units will be required for the most severe cases and for those coming under treatment at a late period. He gives the serum by intramuscular injection into the outer side of the thigh, but in the bad cases part of the dose is given intravenously. Even such large doses as the last named may need to be repeated.

Whether single or repeated doses should be administered is another question of importance. An insufficient first dose cannot be wholly compensated by later injections, and for this reason Park (1921[1]) and others advocate a single primary dose of sufficient magnitude. Other authorities, however, here and elsewhere employ repeated doses, and Friedemann (1922[1 & 2]) considers that the repetition of large doses is an important factor in the cure of severe cases. Madsen (1929, unpublished lecture) stresses the use of a very large single dose, and doubts if repeated doses are of any value. The greater efficiency of large doses over small ones probably rests on the fact that combination of toxin with tissue is not completed at once but is progressive, a state of loose and dissociable combination being present in the early stage, and a massive dose of antitoxin may then dissociate this loose toxin-tissue combination, neutralize the toxin thus liberated, and so prevent the pathological effects which would result from the final stable toxin-tissue combination.

Early treatment is of paramount importance, for it is almost the universal experience that antitoxin acts most efficiently when given early in the attack, and that it becomes less efficient as the moment of first administration is delayed. The following table (Table I) from the *Annual Report of the Metropolitan Asylums Board*, London, for 1908, p. 248, illustrates the importance of early treatment:

TABLE I.

	Day of Disease on which Treatment began.									
	1st.		2nd.		3rd.		4th.		5th and later.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
	202	6	1,076	70	1,182	125	822	106	1,249	185
Percentage Mortality	3·0		6·5		10·6		12·9		14·8	

It is true that statistics of this kind are to be accepted with caution in their entirety, for the milder cases tend to get well at home, and consequently it is the more severe cases that are admitted to hospital 5 or 6 days after onset.

EFFICACY OF ANTITOXIN-TREATMENT IN DIPHTHERIA.

It is difficult to evaluate the efficacy of antitoxin-treatment in diphtheria, for other factors have doubtless contributed to the lowered mortality of the disease which has prevailed during the last thirty years. Such factors include cyclical change in the severity of the disease, general improvement of hygiene and of the social condition of the people, and of the intelligence of parents, improvement in diagnosis, recognition of the importance of treating doubtful cases as though they were diphtheritic, and notification, which leads to earlier removal to hospital and better treatment. Hospital accommodation has also been increased, and the general nursing and treatment of the diphtheria case has also improved as a result of increased knowledge of the disease. Nevertheless there is sufficient evidence of first-rate value, backed up by a mass of statistics of secondary importance, and by a great body of professional opinion, to show that antitoxin-treatment is of the utmost value in human diphtheria.

Clinically, in cases of diphtheria treated with antitoxin the extension of existing, and formation of fresh, membrane are stopped, and that already present clears off rapidly. When the nasal passages are affected, the foul discharge that is so often present quickly ceases. The lessening of the faucial inflammation allows the patient to breathe and to take nourishment without discomfort. The enlargement of the cervical glands and the inflammation of the cellular tissues of the neck subside. A visible improvement in the condition of the patient ensues, pulse-rate and temperature fall and appetite returns.

In pre-antitoxin days the case-mortality was in the region of 30 per cent.: with antitoxin it is in the neighbourhood of 10 per cent. Still more striking is the result in laryngeal cases. From the mass of statistics collected by Goodall (1899) and others it will be found that about 33 per cent. only of all laryngeal cases, whether operated upon or not, recovered in pre-antitoxin days; and of the cases not operated upon about 47 per cent., and of the tracheotomy cases not more than about 29 per cent., recovered. With the use of antitoxin, of all laryngeal cases about 72 per cent. recover, and of the cases not operated upon about 80 per cent., and of the tracheotomy cases about 63 per cent., recover. While these statistics taken by themselves may not be of much evidential value, taken in conjunction with other facts and figures they may be accepted as significant. Thus, at a children's hospital in Paris the case-mortality in the 4 years preceding antitoxin treatment averaged 51·7 per cent. Then for 6 months every case of diphtheria was given antitoxin, all other treatment remaining precisely as before; the case mortality among 448 cases was now 24·5 per cent. At a neighbouring hospital during the same period 520 cases were treated, none of which received antitoxin. Of these, 316 died—a case-mortality of 60 per cent.

Injections of antitoxin are followed in a certain number of cases—20 to 40 per cent. of all cases, according to various estimates—by a disturbance of health of a fairly defined character, known as ‘ serum sickness ’. The symptoms appear 8 to 12 days after the first dose of serum, and consist of rashes, commonly of an urticarial character, with fever and pains in the joints. After lasting for from a few days to a fortnight the disorder usually terminates. Serum sickness is caused by the foreign protein of the horse-serum and not by the antitoxic constituent, for the serum of an untreated horse causes a similar disorder, and the frequency of serum sickness varies directly with the dose of serum administered. The Metropolitan Asylums Board (1928–9) reports that for the same approximate unitage, serum sickness is in all respects—severity of rashes, incidence and severity of pyrexia, and incidence of joint pains, œdema, and nausea and vomiting—more marked with unconcentrated antitoxin, contrasted with concentrated antitoxin.

On rare occasions sudden collapse may follow an injection. It may be of serious or alarming character, even fatal, and is either of an anaphylactic nature, due to sensitization by a previous serum treatment, or is allergic and due to a natural condition of hypersensitiveness.

The Incidence and Spread of Diphtheria.

Diphtheria shows seasonal, periodical, and local variations in incidence. It is more prevalent in autumn and winter, in some years than in others, in urban than in rural areas, and in general the denser the population. The disease is infrequent in the tropics.

FAMILIAL AND AGE INCIDENCE OF DIPHTHERIA.

If diphtheria be introduced into a restricted and closely associated community, such as a family of children, it does not usually spread to all the members exposed to the infection. It has been determined, for instance, that in families of 4 or 5 children, while 2 or 3 may be attacked, it is exceptional for all 4 or 5 to suffer. Assuming that on an average the opportunities for infection of different members of one family are alike, this unequal incidence argues an unequal disposition to the disease.

A similar selective action is seen in the effect of age. Diphtheria is essentially a disease of children, though the infant up to 1 year is rarely attacked, and adults almost escape. The maximum incidence is found in the age groups 2 to 5 years and 5 to 15 years. Thus, in the County of London for the years 1921–5, with a total of 65,000 cases and nearly 4,000 deaths at all ages, 82·5 per cent. of the cases and 96·7 per cent. of the deaths occurred in children under the age of 15, and of the total cases about 25 per cent. occurred in the age group 2 to 5 years and 49 per cent. in the age group 5 to 15 years (Graham Forbes, 1927). Many suggestions were advanced in the past to account for this variable susceptibility of childhood to the disease. Thus, it has been supposed that the infant is isolated to a considerable degree in the home and therefore does not come in contact with chance infections, whereas the opposite condition obtains at school age and hence the greater incidence among the age group 5 to 15 years. Factors such as these are now regarded as being of subsidiary importance, since it has been found that small amounts of diphtheria antitoxin are frequently present in the blood, and that the incidence of diphtheria occurs almost entirely in those whose blood contains little or no antitoxin. The most important contribution to this subject is the work of v. Gröer and Kassowitz (1915, 1917, 1919), who estimated the amount of antitoxin in the blood (1/200 unit or more per c.cm.) by the Römer method (p. 109) in a number of individuals at all ages. At birth, 84 per cent. of infants had antitoxin in the blood. After birth, there is a rapid fall to 32 per cent. at 9 to 18 months, and 28 per cent., the lowest figure reached, at 2 to 3 years. After then, there is a gradual rise in the number of persons with antitoxic properties, to 40 to 50 per cent. at 3 to 7 years and 50 to 60 per cent. at 7 to 14 years, until at the age of 17 to 18 years the number had again risen to 84 per cent. as at birth, at which figure it is afterwards maintained. It is true that these figures were based upon the examination of a particular class, and not upon a sample of the entire normal population, and must not, therefore, be interpreted too literally. Nevertheless they are suggestive, and making allowance for other factors, such as facilities for infection at school age, it will be observed that the incidence of diphtheria runs parallel with the percentage of those without antitoxin in the blood.

Very similar figures have been obtained by the use of the Schick test (p. 130), which consists essentially in the intracutaneous inoculation of a minute amount of diphtheria toxin. In an individual whose blood contains 1/60 to 1/40 unit of antitoxin per c.cm. the dose of toxin is neutralized and no reaction ensues—Schick-negative and immune. If, however, the antitoxin is less than this amount, the dose of toxin is not neutralized and more or less reaction occurs at the site of inoculation—Schick-positive or non-immune. Zdrodowski and Haliapine (1928) Schick-tested 27,000 children at Baiku with the following results at various ages :

At	3 months	5·0	per cent. were Schick-positive.
,,	4–6 ,,	24·6	,, ,, ,, ,,
,,	7–12 ,,	36·8	,, ,, ,, ,,
,,	1–3 years	60·5	,, ,, ,, ,,
,,	4–6 ,,	42·5	,, ,, ,, ,,
,,	7–12 ,,	22·1	,, ,, ,, ,,
,,	11–14 ,,	13·5	,, ,, ,, ,,

These figures indicate that 95 per cent. of young infants are Schick-negative, and, therefore, presumably immune, and that the largest number of Schick-positives or non-immunes occur in the age-group 1 to 6 years, which accords fairly well with experience.

As the majority of adults have antitoxin in their blood, it may be assumed with some degree of certainty that the antitoxin present in the infant is derived from the mother, being transmitted before birth from her blood by the placental circulation, and after birth by her milk. It is known that if antitoxin is present in the blood of the mother it passes into her milk, and the offspring will absorb some of it in spite of digestion. There is some evidence that among older infants at an age when the percentage having antitoxin is much less than at birth or soon afterwards, antitoxin is about twice as frequent among the breast-fed as among the bottle-fed.

After weaning, the number of children having antitoxin in their blood steadily falls as age advances, but after the fourth or fifth year is passed the number rises again and by the seventeenth year some 85 per cent. of individuals have antitoxin in the blood, at which figure it remains. The source of the antitoxin in the adult has been much discussed, but the view now generally accepted is that it results from the immunizing action of obscure infections. The antigen most likely to generate the diphtheria antitoxin would naturally be the diphtheria bacillus itself; in other words, contact with toxigenic *C. diphtheriæ* or its products in the natural environment is the origin of the diphtheria antitoxin present in the blood of man (Dudley, 1928). This hypothesis has much to support it and is the one most widely held. The antitoxin naturally present in man is by all available tests identical with that artificially evoked as a result of the treatment of an animal with diphtheria toxin. Carriers of virulent diphtheria bacilli invariably have a high content of antitoxin in their blood. Dudley (1923) found the Schick reaction negative in the case of 9 carriers of virulent, and positive in the case of 11 carriers of non-virulent, bacilli. That the antitoxin, and the immunity therefrom, depends upon experience of, or contact with, diphtheria bacilli, rather than is a physiological function of age, receives support from Dudley's observations (1928) in a residential school. He found that 33 per cent. of boys over 14 resident for less than a year were Schick-positive, whereas only 16 per cent. of boys under 14 resident for more than a year were Schick-positive. That is to say, immunity, judged by the Schick test, was twice as frequent among younger boys who had greater opportunity for contact with the diphtheria bacillus, than among older boys who had less opportunity for such contact. An hereditary factor may also be concerned in the development of Schick-negativeness (see p. 132).

The question may be asked, Is efficient contact with diphtheria bacilli so prevalent that in the course of years 80 to 85 per cent. of the surviving population will have experienced it? In order to frame some kind of answer, the carrier rate may be estimated. Dudley (1923) found that at different periods 3·5 to 4·7 per cent. of those examined showed bacilli morphologically similar to *C. diphtheriæ*, and of the organisms isolated about 72 per cent. proved virulent. Graham Forbes (1928) states that in 1921 and 1922, out of 9,200 in London schools examined, 656 or 7·1 per cent. showed diphtheria-like bacilli. The organism was isolated from 442 of the positives and proved virulent in 67 per cent. Since 1903, records are available of the diphtheria carrier rate among over 74,000 London school children, showing that it ranges from 4·5 to 10 per cent., averaging 6·3 per cent. per annum. Salck (1927) made during one year 2,105 examinations of 446 children attending the Tübingen Kinderklinik and found diphtheria-like bacilli in 5·4 per cent. of sucklings and youngsters up to 1½ years, and in 8·3 per cent. of all examined. Kliewe and Hofmann (1927) examined 3,272 sucklings and young children and found diphtheria-like bacilli in 185, or 5·7 per cent. Pieper (1928), however, found only 392 carrier cases, or 1·06 per cent., among 36,824 children attending school in Greater Berlin during 1925–7.

If, then, in the course of only one year single individual examinations show the presence of the carrier state among 6 to 7 per cent. of school children, it does not seem unreasonable to suppose that in the course of, say, the 10 years previous to 17 years of age, 80 to 85 per cent. of the population may have been sufficiently pronounced carriers of the diphtheria bacillus to account for the amount of antitoxin found to be present in the majority of adults.

Another problem has, however, to be faced. Is one ' infection ' with the diphtheria bacillus sufficient to generate the antitoxin which is supposed to protect the individual for the remainder of life ? If it is admitted that the general immunity of the adult to diphtheria is due to previous ' infection ' with the diphtheria bacillus, it may reasonably be supposed that an ' active ' immunity results, with an increase in antibacterial processes, such as bactericidal, bacteriolytic, and opsonic ones, which tend to prevent infection and to produce lasting immunity. In this event, the antitoxin present may be only a by-product and an expression of the immune state.

It seems, however, to be generally assumed that the immune state of the adult is due to the antitoxin itself. If this be the case, does the antitoxin produced by a single ' infection ' with the diphtheria bacillus persist throughout life, or are recurring ' infections ' necessary to form the antitoxin which is required for protection during the course of a long life ? Whereas a ' foreign ' antitoxin, like therapeutic antitoxin produced in the horse, is eliminated in the course of 3 or 4 weeks, antibodies produced autogenously persist for a much longer period. This is seen, for instance, in the case of the agglutinin after an attack of typhoid fever, and in diphtheria convalescents antitoxin has been detected in their blood for some months after the attack, but there is no evidence that antitoxin formed as a result of a single ' infection ' will persist for years. Another mechanism exists, however, which tends to promote antitoxin formation. Glenny and Südmersen (1921) found that if diphtheria toxin is injected into an animal not previously treated, a latent period of 3 weeks elapses before antitoxin can be detected and the maximum antitoxin content is not reached until about 2 months after the injection. When, however, the same amount of the same antigen is injected into a previously treated animal, antitoxin appears in the circulation at a far greater rate. The latent period is only 3 days, and the maximum antitoxin level is reached in about 8 days, and is from 10 to 100 times that attained after a first injection in the normal animal. They described these two phases as resulting from the effects of a ' primary stimulus ' and a ' secondary stimulus ', and, further, each succeeding stimulus causes a still greater response. It follows that once an individual has been subjected to the primary stimulus of a definite ' infection ', the smallest ' infection ' or contact with the diphtheria bacillus afterwards will suffice to maintain the immunity, and Glenny (1925) states his belief that men and horses, and possibly other animals, are repeatedly infected to a slight extent with diphtheria. It is conceivable also that stimuli other than the specific antigen may act similarly once antitoxin has been formed in the course of immunization. Thus, the antitoxic potency of the blood of immunized animals may be raised by the injection of manganic chloride (Walbum and Mörch, 1923), and of pilocarpine (Salomonsen and Madsen, 1898). Specific agglutinins in an immunized animal may be increased by the injection of heterologous bacteria (Dreyer and Walker, 1910), or of deuteroalbumose (Fleckseder, 1916), and in individuals inoculated with typhoid vaccine the H-agglutinins undergo a restimulation in the course of various febrile conditions (Felix, 1929).

To sum up this section : immunity to diphtheria runs parallel with the presence of a small amount—1/200 to 1/40 unit per c.cm.—of diphtheria antitoxin in the blood, and is generally regarded as being dependent upon the antitoxin itself. This antitoxin is formed in response to an ' infection ' or contact with virulent *C. diphtheriæ*, such as occurs in a carrier state, but without the production of clinical diphtheria. Judging by the frequency of the carrier state among children of from 3 to 15 years, there is ample opportunity for such ' infection ' to have occurred, so that 80 to 85 per cent. of young adults attain the immune state. Respecting the residue of non-immunes, either the necessary infection has not occurred, or possibly they, or some of them, may not respond to the stimulus which generates the antitoxin. This primary antitoxic immunity would not, however, last more than a few months or a year or two, and for prolonged antitoxic immunity we must postulate recurrent diphtheritic ' infections ', which need not be nearly so intense as the primary stimulus ; it is even conceivable that non-specific stimuli would be effective once the primary specific stimulus has acted. Another factor, which has not received much consideration, is that possibly the primary stimulus may induce a condition of active immunity of long duration, and that the presence of antitoxin is not the cause, or not the only cause, but is merely the expression, of the immune state.

It has been shown that horses not infrequently have natural antitoxin in the blood, and this fact was formerly considered to be a formidable objection to the ' infection ' explanation of the genesis of antitoxin in man—if horses can generate antitoxin without infection by *C. diphtheriæ*, why should not the same mechanism provide the natural antitoxin in man? This objection is no longer tenable since it has been shown that *C. diphtheriæ* may occur in the horse (see Glenny, Vol. VI) and it seems reasonable, therefore, to suppose that the diphtheria antitoxin found in the blood of some horses is due to the specific stimulus of infection by toxigenic diphtheria bacilli just as in man.

PRESENCE OF *C. DIPHTHERIÆ* APART FROM CLINICAL DIPHTHERIA.

Convalescents. The persistence of diphtheria bacilli in the throats of cases after the disappearance of the membrane is well known and may continue for months. When tested, the virulence of the bacilli is generally undiminished, and avirulent strains are seldom observed. Thomson, Mann and Marriner (1929) have investigated the apparent rate of disappearance of diphtheria bacilli after an attack. They find that infection tends to persist longer in the younger patients and when the nose is affected as well as the throat. They give the following table (Table II) showing the percentage becoming negative each week during convalescence for a total of 2,966 cases:

TABLE II.

——	Nose or Nose and Throat.	Throat.
Within 2nd week	298 = 24·0 per cent.	861 = 49·9 per cent.
,, 3rd ,,	159 = 12·8 ,,	255 = 14·8 ,,
,, 4th ,,	184 = 14·8 ,,	254 = 14·7 ,,
,, 5th ,,	158 = 12·7 ,,	138 = 8·0 ,,
,, 6th ,,	136 = 11·0 ,,	86 = 5·0 ,,
,, 7th ,,	97 = 7·8 ,,	46 = 2·7 ,,
,, 8th ,,	52 = 4·2 ,,	41 = 2·4 ,,
,, 9th ,,	51 = 4·1 ,,	18 = 1·0 ,,
,, 10th ,,	45 = 3·6 ,,	15 = 0·9 ,,
,, 11th ,,	32 = 2·6 ,,	5 = 0·3 ,,
,, 12th ,,	28 = 2·3 ,,	7 = 0·4 ,,
	1,240 = 99·9 per cent.	1,726 = 100·1 per cent.

Larval diphtheria. Cases of infection with *C. diphtheriæ* in which the toxic signs of diphtheria are not developed are known by this name. Cases of nasal infection, so-called 'fibrinous rhinitis', skin infections, infections of wounds, and other chronic infections come under this designation. Virulent diphtheria bacilli are present and may escape freely from the subject, who, being 'ambulatory', is a considerable danger to the community.

Contacts and carriers. Those who happen to have contact with cases of diphtheria tend to become carriers of the diphtheria bacillus, and the closer the contact the larger becomes the number of those who carry. A 'carrier' may be defined as a person who harbours in his throat or elsewhere virulent diphtheria bacilli without showing manifest signs of the disease (see also p. 130). Carriers of diphtheria bacilli do not, or very rarely, succumb to diphtheria, presumably because they are protected by the presence of natural antitoxin in their blood. The bacilli present in contacts and carriers are for the most part virulent, and it is exceptional to isolate non-virulent strains. The carrier state in most convalescents is short, averaging some 10 days. Chronic carriers in whom there is prolonged infection become so by virtue of some local condition, such as infection of the tonsils or accessory adenoid tissue.

Animals. Animals play an insignificant role, if any, in the spread of diphtheria. *C. diphtheriæ* has been isolated from the horse (p. 120), and from lesions on the udder of the cow. The diphtherias of animals and birds have nothing in common with human diphtheria.

Fomites, food, &c. Instances have been reported of the presence of the diphtheria bacillus on such articles as a baby's bottle, the bib of a coughing child, and pens and pencils, and on the floor between the beds of diphtheria patients, but they are very exceptional. Although the organism may survive for weeks in the dry membrane or on silk threads, in actual practice it survives only a short period—2 or 3 days—when sprayed into the air, and is not found to any extent even in the air of a diphtheria ward.

Water, sewage, and sewer air play no part in disseminating the disease. Several outbreaks of diphtheria due to the consumption of infected milk have been recorded, the bacilli generally being derived from a human carrier. Occasionally the milk may be infected from diphtheritic lesions on the cow's udder, a recent instance of which is recorded by McSweeney and Morgan (1928).

MECHANISM OF THE DISSEMINATION OF *C. DIPHTHERIÆ*.

In the great majority of cases the actual mechanism by which the bacillus is transferred effectively to a patient must remain conjectural. Now and then there may be an obvious source, e.g. by kissing, by a midwife, or by sucking a tracheotomy wound, but ordinarily it is difficult to incriminate with certainty even a particular carrier.

It is generally inferred that infection is derived from a case or cases of the disease before diagnosis and isolation, from larval cases, convalescent cases, contacts and carriers, and proof being impossible, it is reasonable to suppose that the disease is spread from such carriers by the method of droplet infection.

The possibility of direct infection by ejection of particles of saliva in the act of coughing or even speaking has been recognized for a long time, and is generally accepted not only in diphtheria but in other diseases in which the virus is present in the throat. Whatever deductions may be drawn from experimental studies of the survival of the bacilli in the dust of floors, it is clear that they are found too seldom in such conditions to make infection by dust disturbance probable. According to Teague (1913), the danger of infection depends upon the length of time during which the bacilli ejected by patients remain alive in the air and not upon the ground. The chief lethal factor, in his view, is drying, and this is apt to be slower in ill-ventilated and poorly heated tenement houses than in warm well-ventilated rooms.

It is probable, as suggested by Copeman, O'Brien, Eagleton and Glenny (1922), that the number of bacilli present in the throat, as shown by the profusion of a culture, has an important bearing upon the establishment of diphtheria, on the one hand, or the carrier state on the other. In the case of transient carriers the bacilli are often sparse, and it is easy to assume that in some communities these slight transient infections by droplets may be common and may lead to immunity rather than to disease. Other carriers, however, harbour large numbers of bacilli, and these may be looked upon as being more likely to establish the disease in another person. The point raised by Arkwright (Ledingham, 1913) that the index of dangerousness of a carrier may be the frequency with which the bacilli are present in the sputum is possibly the real determining factor. Arkwright found 24 convalescents with bacilli in the throat, but in only half of these were they also present in the sputum. The experiments of Teague (1913) also bear upon this question. He held plates of Loeffler's medium 3 in. from the mouths of 43 convalescent carriers while they talked or coughed, and in only 28 could he demonstrate diphtheria bacilli on the plates.

In general, conditions which facilitate droplet infection do not differ in diphtheria from what is known in other diseases. Assemblage of susceptible persons in large rooms may be looked upon as most important in accounting for the marked institutional prevalence of this disease (schools, barracks, &c.). In particular the assemblage of susceptible persons in large sleeping dormitories is well calculated to spread the infection. Denny (1900) showed the effect of this factor in a school examined by him. Among 80 boys who slept in single rooms he found one carrier, while among 70 others who slept in two large dormitories there were 15 carriers. The spread of diphtheria in fever hospitals was also ascribed by Pugh (1902) to the collection of children in large wards.

Assuming that a minimal infective dose of bacilli is required to establish infection, this will depend upon the difference between two factors—the rate at which the bacillus is received by the individual and the rate at which it is destroyed in the body (see Dudley, 1923). The rate of reception will partly depend upon distance, for the concentration of organisms will diminish as the square of the distance between donor and recipient. Hence the importance of the distance between beds in sleeping dormitories where persons are exposed to infection for a long time. The same degree of infection in a class-room, for instance, is less important because the time of exposure is shorter.

It is obvious that when direct contact is made between an infected source and a healthy person, the disease may be propagated, but usually as a local lesion at the point of contact. Numerous instances of this form of infection are available, and it is probable that most isolated lesions described as ' cutaneous diphtheria ' or ' wound diphtheria ' are instances of the infection by contagion of a pre-existing lesion of the skin.

Apart from possibly the cow in rare instances, animals have no part in the spread of diphtheria. The cat which used to be incriminated has now been eliminated as a carrier of diphtheria through the work of Savage and others (see p. 95).

RELATION BETWEEN THE VIRULENCE OF *C. DIPHTHERIÆ* AND THE SPREAD OF INFECTION.

The influence of the ' soil ', i.e. the susceptibility of the individual, having been discussed, it is necessary now to consider the specific cause of diphtheria, the diphtheria bacillus, as a factor in the incidence and spread of the disease, and the influence of variations of virulence, of localization and survival, and of methods of transmission to the individual.

The pathogenic action of the diphtheria bacillus is complicated by two factors, those of ' virulence ' and ' toxicity ', for the pathogenic action may result from the microbe alone or from its toxin alone. Virulence denotes the power of a microbe to develop in the body of a living animal and to infect it, toxicity denotes the capacity of a microbe to produce toxin. The two terms are often used as synonymous, and with most other bacteria the two factors cannot be separated, but with *C. diphtheriæ* they are distinct, though probably always in some degree related. A completely non-virulent diphtheria bacillus is probably not toxigenic, but a very virulent bacillus is not necessarily so toxigenic as a less virulent one, and vice versa. Thus, Cary (1917) compared the lethal action of the bacilli from a 24-hour culture with the lethal action of filtered broth cultures 10 days old. His result was as follows :

Strain.	No. of Bacilli required to cause Death.	M.L.D. of Toxin.
Park No. 8	1,930 million	0·0075 c.cm.
No. 20	160 ,,	0·025 ,,
No. 13	1·5 ,,	0·05 ,,
No. 11	1·2 ,,	0·0075 ,,
No. 2059	6·5 ,,	0·5 ,,

Thus, Park No. 8 and No. 11 were of equal toxigenic power, but the former was vastly less virulent than the latter.

Different strains exhibit, therefore, different degrees of virulence, and on general grounds, and relying on the work of Cary, Barratt, and others, it may be suggested that all grades of virulence may exist from the virulent strains killing with a few bacilli, through those killing only with a large number of bacilli, to those which do not kill but produce a lesion. At the same time it is desirable to note that strains of undoubted diphtheria bacilli which produce local lesions without death are extremely rare or possibly do not exist if a sufficient quantity of inoculum is used, and, therefore, it may be held that diphtheria bacilli are either completely non-virulent or are fatal to guinea-pigs, the latter effect, however, being determined by different quantities.

This conclusion is supported by the work of Powell (1923). He found that his strains were either lethal to guinea-pigs or were harmless in any dose. On testing, however, the degree of virulence of his 23 virulent strains by means of intracutaneous and subcutaneous injections of counted suspensions from Loeffler cultures, he found that none was more than 10 times as virulent as another. His strains were, therefore, fairly similar in pathogenic action, though it is, of course, possible that less virulent cultures might have been encountered among larger numbers.

More recently, Okell and Parish (1926) state, as a result of the examination of 1,286 cultures, that they could find practically no evidence of the existence of cultures of intermediate virulence.

The relation between virulence of the bacillus and its power of infecting, and, therefore, its capacity to spread the disease is obscure. A lack of relation between virulence and clinical condition has long been recognized, but examples in which bacilli non-virulent to guinea-pigs have been associated with disease in man are uncommon. No one doubts that *C. diphtheriæ* is the cause of diphtheria and must be present in every case, but the organism cannot always be isolated, or if isolated is not always virulent, e.g. Dudley (1923) found 2 non-virulent strains in 34. Such a result is not to be taken as an instance of bacilli non-virulent to the guinea-pig being virulent to man. It may with reason be attributed to a mixed infection of the throat with virulent and non-virulent bacilli and the accidental selection of a non-virulent colony for test purposes. All modern authors consider that bacilli non-virulent to guinea-pigs are non-virulent to man and are thus of no epidemiological importance, and that a strain virulent to guinea-pigs, in whatever degree, is virulent to man, but not necessarily in the same degree.

Another question is whether a strain of *C. diphtheriæ* may naturally undergo changes in degree of virulence. It has been suggested that the diphtheria bacillus diminishes in virulence as convalescence becomes established, and that on transference to another person virulence is regained and the disease generated, but there is no satisfactory evidence that *C. diphtheriæ* diminishes in virulence as a result of prolonged sojourn in the throat of a convalescent. It has also been supposed that bacteria, particularly streptococci, associated in the throat with *C. diphtheriæ* may increase its virulence, but again there is little or no evidence of this action. It is quite possible that the combined action of the bacillus and of streptococci may be greater than that of *C. diphtheriæ* alone, and that if in such circumstances a slightly virulent strain is enabled to establish a fatal infection in an animal a permanent increase of virulence may result, but there is no evidence that virulence may be established in a formerly non-virulent strain. Similarly, there is considerable difficulty in altering the virulence of a strain by artificial means. Rosenau and Bailey (1925) found no alteration in virulence of virulent and avirulent diphtheria strains by 36 passages on Loeffler's serum, though decrease of virulence has been described following the action of heat or of an acid culture medium, or as a result of the effect of age upon cultures. Slight increases of virulence may be brought about by passage, but there is no evidence that a totally non-virulent strain can be made to show even a slight accession of virulence by artificial means (see also pp. 141–3). The diphtheria bacillus as regards its virulence maintains an extraordinary constancy.

It would appear, therefore, that alterations of virulence play little or no part in influencing the infectivity of *C. diphtheriæ* or in the natural spread of the disease.

The Prevention of Diphtheria.

IMMUNIZATION.

A relative immunity against diphtheria may be obtained by increasing the antitoxin content of the blood by the injection of antitoxin, ' passive immunization ', or by methods whereby the subject himself forms antitoxin or other protective substances, ' active immunization '.

Passive immunization. Immunization by the injection of diphtheria antitoxin was commenced soon after antitoxin was introduced. The immunity produced by the injection does not last much more than three weeks, and this is a serious disadvantage of the method.

From the records, it appears that the symptoms of diphtheria may be prevented in exposed children with a considerable degree of certainty by the injection of some 500 units of antitoxin (for adults, 1,000 units). No doubt the certainty of protection depends upon the degree of exposure, and in any case it is ineffective if the subject is already in the incubation stage of the disease or after a period of some four weeks.

Antitoxin does not prevent infection by *C. diphtheriæ ;* it, however, prevents intoxication and the signs and symptoms of this for a relatively short period after injection. Therefore, it should only be given when the probability of intoxication is great, and when the circumstances are such that the intoxication may not be recognized rapidly when it occurs.

For instance, when an outbreak of diphtheria occurs in a community which is under close medical observation, it would appear more satisfactory to discard prophylaxis by antitoxin in favour of immediate treatment of such persons as become intoxicated. If this attitude is adopted, injection of persons already immune will be avoided.

When the outbreak occurs in a community which is not in close touch with medical observation and when the circumstances are such that the probability of other cases occurring is great, then the use of antitoxin may be considered. Such instances will be confined to communities of children, since adults are so frequently immune naturally that the likelihood of other cases occurring is not so great.

The prophylactic injection of antitoxin has certain objections both practical and theoretical. Active immunization, which is much more permanent and therefore satisfactory, is rendered abortive ; and, further, if a child develops diphtheria after the effect of the antitoxin has passed off, the therapeutic injection may possibly be rendered less effective owing to the presence of precipitins in the blood and, possibly, may be rendered more toxic by the existence of an acquired hypersusceptibility to the horse-serum.

The prophylactic injection of antitoxin should, therefore, be looked upon as a weapon for use in serious emergencies, which are rare, and even then as a palliative which may leave the state of emergency to develop again in three weeks.

The dose of antitoxin is given subcutaneously. Other methods of administering the antitoxin for prophylaxis have also been suggested, as by instillation into the nose.

Active immunization. Attempts actively to immunize man against diphtheria have long been pursued and a variety of substances and of methods have been employed for the purpose. Some of these methods are presumably designed to develop and increase the antibacterial mechanisms of the body, others to increase the antitoxin content of the blood.

For the first named, vaccines of diphtheria bacilli killed by different methods have been used. Endotoxin has been suggested, and Hewlett (1912) was able to protect guinea-pigs against 3 to 4 m.l.d. of living culture with an endotoxin prepared by triturating washed culture at a low temperature. The intracutaneous injection of living virulent culture of *C. diphtheriæ* has been employed by Böhme and Riebold (1924).

For the auto-production of antitoxin, toxin injections have been employed on a small scale only, owing to the fear of producing paralysis, and toxin with antitoxin, or a modified toxin, have now displaced all other methods. The present wide use of toxin-antitoxin mixtures is mainly due to the work of Park (1913^1) and his collaborators. The toxin-antitoxin mixture has to be carefully prepared and standardized on guinea-pigs. It consists of an active toxin which has been kept for some months in order to ripen and stabilize it, and the mixture is so constituted that it contains about 3 L+ doses* of the toxin with 3 units of diphtheria antitoxin per cubic centimetre, which is the ordinary dose. The formula has been modified from time to time, but still contains approximately the same amount of unneutralized toxin as in the older mixtures. The preparation keeps well, but is preferably used within three months of issue.

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*The L+ dose of toxin is that quantity of toxin which mixed with 1 unit of antitoxin and injected into a guinea-pig acts like a single m.l.d. of toxin (see Glenny, Vol. VI).

A few accidents occurred in early days with toxin-antitoxin mixtures. In one instance, the toxin had been mixed with the antitoxin in two portions, with the result that a ' Danysz effect ' followed and the mixture possessed a greater toxicity than normal. In another instance the mixture became frozen, with the result that the antitoxin was precipitated out and the mixture was rendered more toxic than it should have been (see Glenny *et al.*, 1925).

The toxin-antitoxin is given subcutaneously into the arm, the second dose being given in the opposite arm. Three doses of 1 c.cm. are given at intervals of 7 to 10 days. Children who give a plain positive Schick reaction usually show little or no effect as a result of the injection, but others, particularly the ' pseudo-combined ' reactors, frequently experience local tenderness and swelling with constitutional disturbance and temperatures up to 100 to 103° F. for a day or two.

The object of the treatment being to immunize such children as are not already immune, it is preferable to confine it to children who react positively to the Schick test. In general, therefore, this test should be carried out before the injections are given, and invariably six months afterwards in order to determine whether or not the immunization has been successful. In order to diminish the amount of testing, and so expense, the following suggestions due to Zingher may be followed :

(1) Infants under 6 months are unsuitable for immunization, owing to the frequent presence of antitoxin in their blood.

(2) Children between 6 months and 5 years may all be immunized without a Schick test.

(3) Children of 5 years may all be immunized without a Schick test, since most of them are susceptible.

(4) Older children up to 15 years should be immunized only if the Schick reaction is positive. The same applies to older individuals.

If the Schick test carried out 6 months after immunization is still positive, one or two further injections should be given and usually suffice.

Immunity does not develop immediately after the treatment, for the process of protection is a gradual one, occupying at least 3 months, and sometimes 6 to 9 months, and its completion can be judged only by applying the Schick test. In 75 to 90 per cent. of previously susceptible individuals, at least in a ' city ' population, immunization is complete in 3 to 6 months, a small proportion require a second series of injections, and yet fewer a third series before becoming fully protected. A small percentage of individuals may be persistently resistant to immunization (Forbes, 1927).

The duration of the immunity induced by injections of toxin-antitoxin appears to be protracted, lasting for 6 years and probably reaching 10 years, and thus tides over the most susceptible period of life, merging into the naturally acquired immunity of adult years. A caution is necessary in attributing results to toxin-antitoxin inoculation alone, for, as Dudley (1928) has pointed out, immunization through exposure to infection may result in the production of immunity in a short time in a suitable social environment.

It is difficult at present to adduce evidence of the practical value of toxin-antitoxin inoculation in preventing diphtheria—the method has been in use too short a time—but inasmuch as the disease is a rarity in those individuals in whom the Schick test is negative (see p. 133), and toxin-antitoxin injection brings about this state, the inference is that it can hardly fail to be of value. Park and his collaborators state that among 10,000 children who had been under observation for from 3 to 6 years, no case of diphtheria had occurred in any child who had received the full course of toxin-antitoxin and had given a Schick-negative reaction. Fitz-Gerald states (1928) that between October, 1925 and March, 1927, 400,000 persons were injected with anatoxin without mishap. At Hamilton, Ontario, in the pre-injection years 1905–25 the diphtheria morbidity rate varied between 100 and 600, and the mortality rate between 10 and 40 per annum per 100,000 population; in 1927, the corresponding figures were respectively 9 and 0·8, a result difficult to attribute to any factor other than immunization. In a residential school an endemicity of diphtheria equivalent to 5 per cent. per annum dropped suddenly to zero after the inoculation of the Schick-susceptible members with a diphtheria prophylactic (May and Dudley, 1929).

Agents other than toxin-antitoxin mixtures have more recently been introduced for active immunization against diphtheria, in particular toxin modified by the action of heat, of formalin, or of other reagents. Such modified toxin, which is non-toxic, is termed ‘ toxoid ’; it may be regarded as toxin which has lost its toxophore groups, but still retains its haptophore groups and its power of combining with antitoxin. Toxoid and toxoid-antitoxin mixtures (Glenny and Hopkins, 1923) are being much used, and still more recently Glenny and Pope (1927) have advocated toxoid-antitoxin floccules—suspensions of the floccules that form when toxoid and antitoxin are mixed in certain concentrations.

A form of toxoid known as ‘ anatoxin ’, obtained by the action of formalin upon diphtheria toxin, has been introduced by Ramon (1928), and has of late been extensively used abroad as a substitute for toxin-antitoxin, over which it presents the great advantage of being non-toxic, so that an untoward event can hardly happen by its use. The immunity against diphtheria infection conferred by anatoxin has been studied experimentally by Moloney and Fraser (1929), who find that guinea-pigs immunized with anatoxin remain immune for at least a year and a half. Immunization against diphtheria by means of anatoxin has been extensively practised in France (see Martin, Loiseau and Laffaille, 1928), in Belgium (see van Boeckel, 1928), in Germany (see Sdrodowski and Chalapina, 1927), and in Canada (see Fitz-Gerald, 1928), apparently with good results and without accident of any sort.

The Schick test-dose has also been combined with the immunizing agent, so that immunization may proceed while the result of the test is being awaited, and at the same time one inoculation is saved in the case of those who react and would then be immunized (see Glenny and Waddington, 1926).

ISOLATION AND DISINFECTION.

Isolation. Isolation implies the segregation of persons found to be infected with virulent diphtheria bacilli from those not infected until they become free from the infection. In practice this may be very difficult to effect satisfactorily.

The carriers are usually disclosed among contacts of cases of diphtheria by means of mass swabbings of throats, the object being to detect the carrier who has possibly infected the case rather than carriers infected by the case. As there is no evidence that the non-virulent bacillus takes any part in the spread of the disease, it is questionable whether it is justifiable to segregate carriers unless the bacilli present in the throat have been shown to be virulent. The carrier is kept isolated until ' cured '. It is usually required that 2 to 4 consecutive swabbings, taken at intervals of 3 to 7 days, and in the absence of local treatment for several hours previously, shall be negative.

McCartney and Harvey (1928) emphasize the long-recognized fact that the discharge of diphtheria bacilli in carriers may be intermittent, and considers that 6 consecutive negative examinations, taken twice weekly, over a period of 3 weeks, are necessary before a patient should be certified free from infection. When the bacilli persist for 12 weeks the individual may be classed as a carrier.

Harvey (1928[1]) considers that in culture tests for the determination of disappearance of bacilli and freedom from infection, the cultures yielding negative results after 24-hours' incubation should be incubated for a further 24 hours, for as many positives on an average were obtained after the second day's incubation as after the first.

Isolation is likely to be most effective in limited sporadic outbreaks in which the number of infected persons as disclosed by swabbing is small.

Disinfection. The diphtheria bacilli do not exist to any serious extent apart from the immediate vicinity of the patient, and the destruction of diphtheria bacilli upon contaminated objects, though not to be omitted, has, therefore, relatively little importance in preventing the spread of the disease, and terminal disinfection of the dwelling is probably of little value. Contaminated clothing or articles may be rendered free from infection by the usual methods with ease, as *C. diphtheriæ* has a limited resistance to antiseptics and a low thermal death-point. In schools, drinking-cups, pens, pencils, and slates are liable to promiscuous use and infection. If the disease is spread by milk, the source must be discovered and removed.

The *disinfection of carriers* is a problem which has not yet been solved. The value of any treatment for the purpose is difficult to appraise, for carriers naturally tend to free themselves of bacilli without special treatment according to a definite mathematical sequence, viz. a constant reduction in the number of positive cases daily approximating to 5 per cent. of their daily number (see also p. 121).

The methods of treatment that have been employed include antiseptic treatment with sprays and gargles, local specific treatment by the use of powders or pastilles consisting of an *antibacterial* diphtheria serum (antitoxic serum is of no use whether locally or by injection). General specific treatment has been tried in various forms, e.g. toxin, toxin-antitoxin and endotoxin. The production of a local inflammatory reaction in the throat by means of cultures or suspensions of *Staphylococcus aureus* or other agent has also been tried. Surgical treatment by removal of tonsils and adenoids seems to be more adequately supported than other methods (see Harries, MacFarlane and Gilhespy, 1927 ; McCartney and Harvey, 1928).

Harvey (1928[2]) states that mild alkaline nasal douches yield excellent results in the case of *nasal* carriers. Ultraviolet rays and X-rays are stated to be successful in the treatment of carriers by Stewart (1923) and Kahn (1924) respectively.

Harvey (1929) finds that the throat flora of carriers does not differ materially, quantitatively or qualitatively from that of non-carriers. In nasal carriers the bacterial flora is the same as, but is more abundant than, in non-carriers.

The Schick Test.

The Schick test or reaction, introduced by Professor Bela Schick, of Vienna, in 1913, consists in injecting intradermally a minute dose of *diphtheria toxin. This gives rise to a local reaction at the site of inoculation* in individuals unprotected by the presence of natural antitoxin, whereas if antitoxin is present, the toxin is neutralized and no reaction follows the injection. The Schick test reveals the presence of about $\frac{1}{60}$ to $\frac{1}{40}$ unit of antitoxin per c.cm. of the person's serum, and a case that does not react to the injection of toxin is assumed to have at least this amount of antitoxin present. It has been established that the presence of antitoxin in a person's blood in a quantity of $\frac{1}{50}$ to $\frac{1}{20}$ unit is a considerable preventive against an attack of diphtheria. The Schick test reveals, therefore, the fact of the susceptibility or otherwise of an individual to diphtheria toxin, and, therefore, his susceptibility to the disease. It is thus applicable for indicating those members of a community who require to be immunized if there be risk of infection. It may also be used to differentiate carriers of diphtheria bacilli from cases of diphtheria, for the former react negatively, while the cases will react positively.

The test toxin. The dose of toxin adopted is $\frac{1}{50}$ m.l.d., because this amount is the largest which can be used without producing reactions in persons who have sufficient antitoxin in the blood to give relative immunity, and if smaller quantities be used some cases will give negative reactions although they have too little antitoxin to provide immunity. Accuracy in the preparation of the test toxin is therefore essential, and the test is best carried out with materials obtained from a trustworthy source in the form of outfits which merely require mixing as directed in order to furnish the requisite dilutions (for mode of preparation, see Glenny, Vol. VI). When required for use in the test the stock toxin is so diluted that $\frac{1}{50}$ m.l.d. is contained in 0·2 c.cm. A primary dilution may be made with saline so that each cubic centimetre contains 10 m.l.d.; this dilution remains active for a fortnight. This primary dilution diluted one hundredfold will contain the required dose of $\frac{1}{50}$ m.l.d. in 0·2 c.cm. Part of the diluted toxin is heated to 70° C. for 5 minutes and is used as a control. The heating destroys the specific toxin, but does not injure the other constituents of the toxin broth to which the 'pseudo reactions' are due.

The diluted toxin is unstable and should not be kept more than a few hours before use.

The test needs care and practice to carry it out properly. A small well-fitting syringe (1 c.cm.) provided with a fine sharp needle $\frac{1}{4}$ inch long is employed, one syringe being used for the toxin and another for the control. The injection of the toxin and of the control is made into corresponding areas of the flexor aspect of the forearms, the skin having been cleaned with a little spirit. The needle is introduced into the epidermis with the bevel up and is passed in parallel to the surface and so superficially that the bevel can be seen through the layers of the *stratum corneum*. The requisite quantity (0·2 c.cm.) is then injected with some little pressure, and a small white wheal will rise, its surface pitted where it is anchored down by the hair follicles and sweat ducts. No dressing is required.

The types of reaction which may be observed are four in number:

1. Negative.
2. Positive.
3. Pseudo (or negative and pseudo).
4. Combined (or positive and pseudo).

1. The negative reaction is recognized by an entire absence of reaction in both arms.

2. In the positive reaction nothing is observed on the control arm at any time, but on the test arm after 24 to 36 hours a red flush begins to develop and is at its maximum in 4 days. At this time it is a round circumscribed area measuring 1 to 2 cm. in diameter and slightly swollen. From its maximum it slowly fades in a further 7 to 10 days to a circumscribed brownish tint with desquamation of the epidermis. The pigmentation may remain for weeks.

3. The negative and pseudo reaction, as is to be expected from an allergic reaction, develops rapidly in 24 hours *equally on both arms.* It is

a red flush less circumscribed than the positive reaction. By the fourth day it has mostly disappeared, often, however, leaving behind a reddish or brownish pigmentation with a certain amount of desquamation.

4. The positive and pseudo reaction is a complex of both positive and pseudo reactions. The pseudo effect develops rapidly on both arms, and as this fades the true positive emerges on the test arm. In this way the test arm tends to acquire the characters of the true positive, while the control arm is clearing up. When both reactions are still present on about the third day, that on the test arm is larger, either obviously or slightly.

If it is only possible to take a reading upon one occasion this should be done not sooner than the fourth day, and not later than the seventh. In most cases the pseudo reaction will have died out in this time.

The conclusions drawn from these results are as follows :

Negative and negative and pseudo = patient is immune.
Positive and positive and pseudo = patient is susceptible.

Certain features of the Schick reaction in connection with sex, race, and heredity may be briefly noticed. The percentage of positive Schick reactors is always greater in the female than in the corresponding male age-group. Reports of Schick testing indicate that the natives in the tropics nearly always possess a higher degree of Schick immunity than is commonly found among Europeans and North American whites. Though clinical diphtheria is infrequent in the tropical zone, diphtheria bacilli carriers seem just as common in many parts of the tropics, e.g. India, as in the temperate zone (see Dudley, 1929).

The potentiality to form antitoxin is probably inherited like other properties of the germ plasm. Zingher's law has been adduced as evidence of an hereditary factor, but according to Dudley is better explained by the acquired immunity hypothesis. Zingher's law states that in general all the members of a family have the same Schick reaction ; when both positive and negative reactors are present, it is the younger members of the family who are Schick-susceptible.

Schick susceptibility seems to depend in some degree upon the blood-group of the individual. Thus, Hirszfeld, Hirszfeld and Brokman (1924) found that when both parents are Schick-negative, the children are generally Schick-negative, when both parents are reactors, all the children are also reactors, but when one parent is susceptible and the other immune, the children of serological group the same as that of the susceptible parent are always susceptible, whereas the children with the group of the immune parent are mostly immune. Hirschfeld (1927) by blood-grouping and Schick-testing individuals by families likewise found that the potentiality to form antitoxin seemed to be inherited with the blood-group. Observations among the Eskimos by Heinbecker and Irving-Jones also suggest an hereditary factor at work, for they found that 37 out of 49 individuals of various ages were Schick-immune, although there was no history of anything resembling a diphtheria epidemic. Dudley (1929), however, points out that all the children under 12 tested were Schick-positive, and all subjects over this age were immune, suggesting that some intense antigenic stimulus of short duration had occurred 12 years previously. As is well known, Eskimos are very susceptible to diphtheritic infection if this is introduced among them.

The Schick test has been extensively employed all over the world, and in Great Britain during 1921–6, some 54,000 persons were tested, of whom 26,400 or 49 per cent., reacted positively. Taking the general population only—mainly children under 15 years—of 24,983 tested, 17,223, or 69 per cent., reacted positively (Forbes, 1927).

The question of the occurrence of clinical diphtheria in those who have proved Schick-negative on testing is one to which much attention has been directed. The evidence available appears to show unquestionably that true *clinical* diphtheria is very rare in genuine Schick-negatives. A small percentage of Schick-negative persons relapse into the Schick-positive condition, and there is obviously no *a priori* reason why these should not be susceptible to diphtheria. Parish and Okell (1928) found that of 533 children who were Schick-negative on the original test made from 1 to 7 years previously, 98·9 per cent. were still clearly negative, and 1·1 per cent. showed a reaction which was read as positive. O'Brien, Okell and Parish (1929) state that only 18 cases of diphtheria were met with during 6 years among a Schick-negative group numbering over 20,000, and in every case but one the disease was mild and diphtherial toxæmia in the clinical sense was absent. The negative response may not always mean *complete* clinical immunity, as for example among the members of a fever hospital staff who may be exposed to virulent infection in massive doses, and Neale (1928) records two instances in nurses. Both nurses were originally Schick-positive, had been immunized with toxin-antitoxin, and were afterwards Schick-negative : the disease in both cases was benign and the membrane thin and filmy.

The occurrence of diphtheria in an individual who some time previously was Schick-negative does not necessarily mean that he is negative at the time of attack. Three conditions should be fulfilled before the diagnosis of true clinical diphtheria in a Schick-negative reactor can be accepted, viz., the clinical appearance must be typical of diphtheria, diphtheria bacilli must be isolated and proved virulent, and the antitoxin content of the individual's blood before administration of antitoxin ought to be determined.

The 'safety diagnosis' of diphtheria occasionally made in Schick-negative individuals harbouring virulent bacilli, but otherwise being little abnormal, is not justified, for they are properly to be regarded as carriers, and carriers with virulent bacilli in the throat are always Schick-negative and are immune to the disease (Okell and Parish, 1926).

Diphtheroids.

Organisms morphologically resembling more or less closely the diphtheria bacillus are known as 'diphtheroids' and are placed in the

Genus *Corynebacterium* with *C. diphtheriæ*, the prototype of the group. Some diphtheroids are met with in man, others in animals, and a few are pathogenic to the lower animals. The investigation of diphtheroid forms in general has been necessary in order to establish the means for the accurate diagnosis of *C. diphtheriæ* in routine practice, and even now the relation, if any, of what is known as the non-virulent *C. diphtheriæ* to the virulent form has not been completely solved. *C. diphtheriæ* stands apart from practically all its congeners by its capacity to elaborate, both *in vitro* and *in vivo*, a potent toxin, on which its pathogenicity for man and animals mainly depends. This property of virulence for certain laboratory animals is so important and decisive that a diphtheroid organism from a human source, which is even moderately endowed in this respect, may with almost complete confidence be diagnosed as *C. diphtheriæ*.

The term ' diphtheroid bacillus ' connotes strictly an organism which possesses a morphology recalling more or less that of *C. diphtheriæ*, but the characters of the group need to be closely defined lest organisms be described as ' diphtheroid ' on meagre and loose evidence. In the Medical Research Council's Monograph, *Diphtheria* (1923), the following definition of *Corynebacterium* is given, which may well be adopted: *Gram-positive rod-like forms, arranged usually in a palisade, not acid-fast, often with club-shaped swellings at the poles, generally with irregularly stained segments or granules, non-motile, without endospore formation, growing aerobically or under micro-aerophilic conditions but often capable of anaerobic cultivation, never forming gas in carbohydrate media, in which they may or may not produce acidity ; they may or may not liquefy gelatin or serum.*

The early investigations led to the isolation of diphtheroids, some of which appeared to possess characters sufficiently well defined to merit the coining for them of specific names, which have persisted, but with further investigation it has become apparent that a multiplicity of forms exists, the characters of which cannot at present be sufficiently differentiated to separate them into good species—forms comparable to those met with among other organisms, for example, the streptococci and micrococci.

The different diphtheroids show much variation in morphology, and may be classified according to their length into (1) very short forms, (2) short forms, and (3) forms of medium length. When stained, some forms stain almost uniformly, others are more or less segmented, and the same variation occurs as regards granules and involution forms. Neisser's method may or may not show granules. Pigment production is the exception, but yellow, orange, brown and pink growths are occasionally met with.

The diphtheroid organisms are almost entirely parasitic on man and animals, and do not appear to occur in nature as free-living saprophytes. They are found in many situations in the body and have been recovered from various obscure diseases in man. It is probable that they are not the causal agents in the majority of these conditions, and that they must be regarded as simple contaminations derived from the skin and elsewhere ; absolutely healthy tissues are known occasionally to have yielded diphtheroid organisms. The group as a whole is singularly devoid of pathogenic properties.

. DISTRIBUTION OF DIPHTHEROIDS IN MAN AND ANIMALS.

In the *throat*, diphtheroids other than *C. diphtheriæ* and *C. hofmanni* (p. 138) are comparatively rare, though others do occur (p. 106).

Nose and nasal sinuses. C. coryzæ segmentosum (p. 143), or organisms simulating it, are the special diphtheroids of this region. Diphtheria-like bacilli have long been known in atrophic rhinitis.

In the healthy *ear*, diphtheroids named *C. ceruminis* and *C. auris* (Graham-Smith, 1904) are quite common : the former is non-saccharolytic, the latter ferments glucose and maltose.

In the *eye, C. xerosis* (p. 143) or forms simulating it are common, and others also occur.

In the *skin*, diphtheroids are common particularly when it is the site of chronic lesions. One type, *C. acnes* (p. 144), has its habitat in the sebaceous follicles, *C. hofmanni* is met with, and three or more forms are frequent in wounds, the commonest of which ferments glucose and saccharose only (Douglas, Fleming and Colebrook, 1920 ; Adami *et al.*, 1918). Diphtheroids have also been isolated from leprous lesions of man and of rats. It has been suggested that they are non-acid-fast stages of the acid-fast mycobacteria of these diseases, but it is probable that they were simply contaminating diphtheroids derived from the skin and subcutaneous tissues.

Diphtheroids are common in the *urine and genito-urinary tract*, and may resemble *C. diphtheriæ* so closely as to lead to error ; they have also been isolated from the *brain, cerebral abscesses*, and the *cerebrospinal fluid.*

In cases of generalized infections diphtheroid organisms have sometimes been isolated, e.g. by Gundersen (1925), from the blood in three cases of obscure remittent fever (*xerosis* type).

Vaccinia. Calf lymph is a fertile source of diphtheroids, and some, e.g. Klein's *B. xerosis variolæ* (1897), have even received names.

Diphtherias of animals. The causative agents of the so-called diphtherias of pigeons, fowls, and calves do not belong to the genus *Corynebacterium*, but diphtheroids may frequently be recovered from the throats of pigeons suffering from avian diphtheria and also from healthy pigeons (Macfadyen and Hewlett, 1900), and Graham-Smith (1904) gave the name *B. diphtheroides gallinarum* to an organism which he isolated from the throat of a healthy fowl. He also isolated a diphtheroid from the throat of a cuckoo. These organisms may resemble *C. diphtheriæ* very closely but are avirulent for guinea-pigs.

Klein (1903) isolated a diphtheroid (*B. muris*) from the pneumonic lung of a rat. It resembled *C. diphtheriæ* in morphology and formed acid from glucose. Inoculated into rats it produced a local lesion only, and it is questionable, therefore, if it was responsible for the natural disease in the rat. Another diphtheroid (*B. renalis bovis*) was isolated from the pus of a renal abscess in cattle, but it is now regarded simply as an associated organism.

Diphtheroid bacilli are often met with in *milk, butter* and *cheese*.

CLASSIFICATION OF THE DIPHTHEROID ORGANISMS.

Various attempts have been made to classify the diphtheroid organisms by means of cultural, fermentation and serological tests. The chief studies on the subject have been those of Morse (1912), Priestley (1911), Mellon (1917) and Eberson (1918) ; these are discussed in some detail in the M.R.C's Monograph, *Diphtheria* (1923).

It is frequently difficult to obtain a diphtheroid organism in pure culture, and a contamination may maintain itself persistently through a long series of cultures though not evident, and this fact in part explains anomalous reactions that are sometimes obtained in the investigation of diphtheroids, notably in the case of *C. diphtheriæ.*

Since 1923 other surveys of collections of diphtheroids have been made with a view to classification. Pesch (1924) examined 190 strains derived from the throat and nose, eye, skin, tuberculous fistulas and elsewhere. He divided them into six principal groups on a basis of morphology and granulation, cultural characters on various solid culture media, and action upon glucose and saccharose. He remarks that it is not sufficient to classify diphtheroid organisms into diphtheria bacilli and pseudo-diphtheria bacilli, and that ' a large number of more or less distinct species of Corynebacteria exist '. Kliewe (1926–7) discusses the variability and grouping of the diphtheria bacillus and other Corynebacteria, 130 strains of which were examined. The characters investigated were morphology and staining, growth on sodium oleate agar and in broth, fermentation of glucose, lævulose, maltose and saccharose, hæmolytic power, agglutination with a diphtheria serum and pathogenicity. The diphtheria bacilli, Group i, are divided into six subgroups, a to f, and according to their activities are termed minus- and plus-variants ; some of these would not be acceptable to us because they ferment saccharose. Group ii contains the pseudo-diphtheria bacilli, and is divided in the same way into five subgroups, a to e.

Barratt (1924) examined the fermentation reactions of a large series of diphtheroids the sources of which were known (see Table III). The ' other sources ' include strains from the eye, cerebrospinal fluid, genito-urinary tract, and elsewhere. One strain came from an oyster and was exceptional as being the only organism in the whole series that liquefied serum. For comparison, 102 strains of virulent, and 31 strains of non-virulent, diphtheria bacilli were also examined, together with

C. pseudo-tuberculosis, ovis and *murium*. The 150 diphtheroids are thus divided into 11 groups, Group v being subdivided into two by the action on maltose. The facts that emerge from this investigation are (1) the multiplicity of groups into which the diphtheroids become divisible, (2) the rarity with which dextrin, mannitol and lactose are fermented, (3) the constancy of the reactions of the virulent and avirulent diphtheria bacilli; with the possible exception of that with dextrin, all strains gave constant reactions, and dextrin was shown to be a substance of uncertain composition (see also p. 81), (4) biochemical grouping bears no relation to source. Thus, urethral diphtheroids are found in Groups iii, vii and xi, and those from the eye in Groups i, ii, iii and xi. Many types, probably distinct species, of diphtheroids occur in a group; for example, Group xi, the non-fermenters, contains several types besides *C. hofmanni*.

TABLE III.

	Source and No. of the Diphtheroids in the Groups.				Fermentation Reactions.						
Groups.	Total.	Nose and Throat.	Ear.	Other Sources.	Glucose.	Maltose.	Galactose.	Saccharose.	Lactose.	Mannitol.	Dextrin.
I ..	21	13	4	4	+	±	+	+	—	—	—
II ..	16	13	1	2	+	±	—	+	—	—	—
III ..	19	10	2	7	+	+	—	—	—	—	—
IV ..	9	7	0	2	+	±	—	—	—	—	—
V ..	13	9	2	2	+	+	+	—	—	—	—
V*a* ..	2	2	0	0	+	±	—	—	—	—	—
VI ..	7	7	0	0	+	+	+	+	+	—	—
VII ..	8	3	0	5	+	+	—	+	—	—	—
VIII ..	11	10	1	0	+	+	+	+	—	—	—
IX ..	1	0	0	1	+	+	+	+	+	—	+
X ..	1	1	0	0	+	±	+	+	—	+	—
XI ..	42	33	4	5	—	—	—	—	—	—	—
Totals ..	150	108	14	28							
C. diphtheriæ (virulent and avirulent)					+	+	+	—	—	—	—
C. pseudo-tuberculosis ovis					+	+	?	—	—	—	—
,, ,, *murium* ..					+	+	—	+	—	—	—

+ = Acid production with or without clot.
± In maltose column = transient acid production.
— = no acid production.

The latest attempt to classify the diphtheroid organisms is that of D. Thomson and R. Thomson (1926). The diphtheroids examined (72 varieties) were divided into five primary groups by the cultural characters on testicular infusion peptone glucose agar. These primary groups are then subdivided into ' types ' by the fermentation reactions with glucose, maltose, galactose, saccharose, lactose, mannitol and dextrin in Hiss's serum-water. The morphological characters and virulence were also determined. The five primary groups, with the number of fermentation ' types ' in each, were as follows :

Group i. Non-chromogenic, profuse growers, aerobic or facultatively anaerobic (22 strains) ; 13 types.

Group ii. Non-chromogenic, profuse growers, anaerobic (4 strains) ; 4 types.

Group iii. Non-chromogenic, delicate growers, aerobic or facultatively anaerobic (25 strains) ; 18 types.

Group iv. Non-chromogenic, delicate growers, anaerobic (5 strains) ; 3 types.

Group v. Chromogenic, profuse growers, aerobic or facultatively anaerobic (16 strains) ; 11 types.

D. and R. Thomson maintain that under the standard conditions stated this grouping is distinct, constant, and valid. For differentiation of varieties, they employ photographs of the films, growths, and colonies, also under standard conditions, which they consider as important a method as any other for the identification of bacteria, as well as supplying a simple permanent record of such characters as can be photographed. They have not attempted to give names to their strains, anticipate that many more will be found, and ' believe that in all probability there are some hundreds of species of diphtheroids '.

The Pseudo-Diphtheria Bacillus of Hofmann.

Nomenclature. The name ' pseudo-diphtheria bacillus ' was coined by v. Hofmann-Wellenhof in 1888 to designate a human diphtheroid which though possessing some resemblance to, presented points of difference from, the diphtheria bacillus and was non-virulent to the guinea-pig. Loeffler also probably met with the same organism, but he appears in addition to have isolated forms which would now be regarded as being non-virulent diphtheria bacilli. The subject is complicated by the fact that about the same time Roux and Yersin applied the term ' pseudo ' to a diphtheria-like, avirulent organism giving an acid reaction in broth and clearly a non-virulent *C. diphtheriæ* in the modern sense. The French school continued to use this terminology until a dozen years or so ago, whereas the British and others from the close of the last century onwards applied the name ' pseudo ' to a form corresponding, so far as can be determined, with von Hofmann's organism, and now commonly known as the Hofmann bacillus. Bergey *et al.*, in the first edition of the *Manual of Determinative Bacteriology* (1923) named this organism *C. hoffmannii*, but the double ' f ' is clearly incorrect and the second ' i ' seems redundant. In the second edition of this *Manual* (1925), Bergey adopts *C. pseudodiphthericum* as the scientific name, and *pseudodiphthericum* certainly has priority as the specific name, as it is used by Flügge (1896), Migula (1900) and Chester (1901). In the M.R.C's Monograph, *Diphtheria*, *C. hofmannii* was the name adopted, presumably following Bergey's first edition, but here we shall use *C. hofmanni* or Hofmann bacillus.

C. hofmanni: Morphology. In its typical form, *C. hofmanni* is a short, straight, somewhat ovoid, rod with rounded ends, having an average length of 1·3 to 1·8μ. It is thicker in proportion to its length than *C. diphtheriæ*, and it possesses a single median unstained transverse septum; the appearance of the stained organism has been well likened to that of a pair of suppositories placed base to base. It maintains its short squat form very persistently in both primary and secondary cultures, and unlike *C. diphtheriæ* never shows any long forms, nor any clubbing or other involution forms, with rare exceptions, e.g. in glucose media. It stains intensely with the Gram stain, which it retains more persistently than *C. diphtheriæ* on treatment with alcohol, and it stains regularly and deeply with methylene blue, without barring, polar staining, or metachromatic granules. The Neisser stain is negative, though now and then an indication of very minute granules may be seen.

C. hofmanni is easily cultivated, and the growths resemble in many respects those of *C. diphtheriæ*, though are generally more luxuriant. It is aerobic and refuses to grow under strict anaerobic conditions.

Growth on serum. On serum *C. hofmanni* grows rapidly and produces whitish soft colonies larger than those of *C. diphtheriæ*.

Growth on agar. Here it grows luxuriantly, forming a white shining moist growth.

Growth in broth. *C. hofmanni* causes a general clouding of the medium. Later the cloud settles to a fine granular deposit.

Growth in stab culture. *C. hofmanni* produces, as a rule, little or no growth in the track but a profuse growth on the surface of the column, while *C. diphtheriæ* produces little on the surface but a very definite track growth. The stab test, however, cannot be absolutely depended upon to distinguish *C. hofmanni* from *C. diphtheriæ*.

Growth on gelatin. A white shining growth develops in the course of a few days without liquefaction.

On *alkaline potato* a distinct yellowish white growth or colonies form, whereas *C. diphtheriæ* gives a thin invisible growth.

In *litmus milk* a well marked alkaline reaction develops in a few days.

Carbohydrates, &c. *C. hofmanni* is sharply distinguished from most other diphtheroids by not fermenting any of the ordinary fermentable substances. Old broth cultures give an indole-reaction, due, however, to indole-acetic acid and not to indole, in this resembling *C. diphtheriæ*.

Pathogenicity. *C. hofmanni* is avirulent to the guinea-pig. Salter (1899) claimed to have shown that it is virulent to small birds. It forms no toxin.

Occurrence and relations. *C. hofmanni* is of frequent occurrence in the throat and less so in the nose, often grows in the primary cultures from swabbings taken in cases of suspected diphtheria, and is the diphtheroid which has in particular to be distinguished from C. *diphtheriæ* in these circumstances. It is likewise frequent in swabbings from diphtheria contacts and may be met with in the eye and skin and other situations.

It has been surmised in the past on various grounds that C. *hofmanni* is an attenuated form of C. *diphtheriæ*, and Hewlett and Knight (1897) and Salter (1899) claimed to have converted the former into the latter by passage through guinea-pigs or small birds. Similarly, many workers, including Hewlett and Knight, claimed to have transformed a typical virulent C. *diphtheriæ* into an avirulent Hofmann bacillus. These views lack confirmation at the hands of other workers, and a large amount of experimental work is opposed to them. Thus, Petrie and others showed that immunization with C. *hofmanni* confers no protection against virulent C. *diphtheriæ*, that inoculation of animals with filtrates of C. *hofmanni* produces no antitoxin, and that such filtrates contain no bodies capable of combining with diphtheria antitoxin. Serologically, the two organisms have no relationship. Thus, Bull and McKee (1924) carried out complement-fixation tests upon three strains of the Hofmann bacillus, three strains of avirulent, and five strains of virulent, diphtheria bacilli, using potent antigens and immune sera. The three Hofmann strains were easily distinguished from the eight diphtheria strains. Bailey (1925) prepared agglutinating sera for avirulent diphtheria, Hofmann and Xerosis strains, but found no relationship between the Hofmann and the other strains. Rosenau and Bailey (1925) made use of the Schick test : guinea-pigs were immunized with the same organisms and were then tested with the Schick reagent, with the result that the Schick test was positive for all the animals, i.e. not a trace of diphtheria antitoxin had been produced.

Mõhrke (1926) considers that the reported conversion of pseudo-diphtheria into virulent forms is not sufficiently well founded to be accepted, and found that a polyvalent diphtheria serum which agglutinated all diphtheria strains tested in greater or less degree did not agglutinate any of eight different strains of diphtheroids.

The negative results of these immunization and other tests have been generally regarded as proving absence of relationship between C. *diphtheriæ* and the Hofmann bacillus, as they seem to do. But this is not a certain criterion, for on the same grounds the non-virulent C. *diphtheriæ* would similarly be regarded as having no relationship with virulent C. *diphtheriæ*, as it reacts negatively to the tests named just as C. *hofmanni* does.

Some recent work, however, once more suggests the possibility of transforming the virulent C. *diphtheriæ* into 'pseudo' forms. Thus, Killian (1924) asserts that by feeding guinea-pigs and mice with diphtheria culture and recovering the organism from the cervical and mesenteric glands, a virulent C. *diphtheriæ* is converted into a 'pseudo' form. Levinthal (1926) making use of cultures derived from single-cell isolations asserts the transformation of a virulent C. *diphtheriæ* into strains conforming to the Hofmann or Xerosis types in morphology, staining, biochemical reactions, and result of animal inoculation (*a*) by a technique consisting of culture in normal rabbit serum massively inoculated from blood-agar plates, and subsequent recovery from the serum on blood-agar plates under particular conditions, and (*b*) by recovery from inoculated animals (vide Killian, above). Gundel (1927) also confirms Killian's work on the recovery of ' pseudo ' forms from guinea-pigs fed with diphtheria culture, and states that a ' pseudo ' bacillus cultivated in a series of dextrose peptone waters becomes diphtheria-like in morphology though continuing avirulent.

Gins and Jermoljewa (1928) state that the addition of fresh organ pulp to nutrient agar influences the growth of the diphtheria bacillus, liver and kidney enhancing growth, whereas spleen is generally inhibitory, as is also fresh bile. In the liver and kidney nutrient medium, a typical diphtheria bacillus within 24 hours changes its morphology to that of the Hofmann bacillus, but in spite of this striking change in form the toxicity remained undiminished. By cultivation in spleen agar, in glycerin broth at 20° C., and in media with the addition of bile, these authors state that the diphtheria bacillus may be converted into an atoxic form, though these atoxic strains would produce active immunity in guinea-pigs. Although alleged change of type of an organism by inoculation into and recovery from an animal must always be suspect, the other work of Levinthal and of Gundel cannot be altogether ignored, but requires confirmation before it can be accepted.

C. hofmanni is an organism characterized by considerable constancy in its characters. Different serological types may exist, just as there are serological ' types ' of *C. diphtheriæ* itself, it may even include more than one species. In Bull and McKee's work mentioned above, one Hofmann strain reacted much better with its own serum than with the sera of the two other strains.

Avirulent Strains of C. diphtheriæ.

The relationship of the avirulent to the virulent *C. diphtheriæ* remains unsolved. These avirulent forms agree in morphological, cultural, and biochemical characters with, and their sera may agglutinate, the virulent organism (Bailey, 1925), and it seems difficult not to believe that they are attenuated forms of *C. diphtheriæ*. But avirulent strains fail to produce immunity against virulent *C. diphtheriæ*, they fail to produce even a trace of diphtheria antitoxin judged by the Schick test (Rosenau and Bailey, 1925), there is no change of virulence in either the virulent or the avirulent strains by cultivation through 50 generations, nor gradations of virulence between the two (Powell, 1923, and Rosenau and Bailey, 1925), and there is little experimental evidence that either form can be converted into the other.

Arkwright (1909) claimed to have produced antitoxin with an avirulent strain of *C. diphtheriæ*, of which 5 c.cm. doses of 2-day- and 8-day-old broth cultures failed to kill a guinea-pig. A horse inoculated with a long series of increasing doses—up to 1,000 c.cm.—of the filtrate of a broth culture yielded at the end of the treatment serum containing 25 to 30 units of antitoxin per c.cm., the initial amount being 0·25 unit. The writer is unaware that this important observation has been confirmed ; it would be worth repeating with adequate controls.

Crowell (1926) records the separation of an avirulent strain from a highly virulent culture of *C. diphtheriæ*. A single cell was isolated from the stock culture by the Ørskov method. Six transplants of this ' parent-strain ' proved virulent. Two other single diphtheria cells were then isolated from the sixth transplant of the parent-strain, daughter-strains ' one ' and ' two '. Daughter-strain ' one ' possessed virulence equal to that of the parent-strain and of the stock culture. Daughter-strain ' two ' was entirely different—all virulence tests were negative, and all attempts to render it virulent failed ; it remained persistently avirulent and atoxic. Six other single diphtheria cells were isolated from the parent-strain but all were virulent. These observations suggest that a virulent strain of *C. diphtheriæ* from time to time ' throws ' an avirulent form, and it is not unreasonable to suppose that this avirulent diphtheria cell may under certain conditions be perpetuated as such, and by selection and isolation ultimately give rise to an avirulent strain. On this hypothesis, there is no actual conversion of the virulent into the avirulent form, the latter arising as a sport or mutant. Such an hypothesis of the derivation of the avirulent form of *C. diphtheriæ* seems to accord with the facts and to afford an explanation of its relation with the virulent organism better than any other so far advanced.

The occurrence of avirulent cells in a virulent culture does not appear, moreover, to be unique and restricted to the diphtheria bacillus, for a precisely similar observation is recorded by Bengtson (1924) in the case of *B. botulinus* (see Vol. III). A toxic single cell isolated from a toxic culture gave rise to toxic and atoxic daughter-cells, and a daughter toxic strain again to toxic and non-toxic substrains, and as in Crowell's observations, non-toxic strains never gave rise to toxic ones.

Crowell's work also receives confirmation from that of Cowan (1927). She obtained definite ' rough ' and ' smooth ' colonies from the Park No. 8 and another virulent strain after repeated plating on serum agar. Strains from the ' rough ' colonies proved to be non-virulent, while those from the ' smooth ' colonies were virulent like the original cultures. Guinea-pigs immunized with the ' rough ' strains were not protected against the virulent strains, continued to be positive to the Schick test, and their serum gave no complement fixation, using the virulent Park No. 8 strain as antigen. Parker (1928) observed similar differences in virulence of ' rough ' and ' smooth ' strains.

One more observation bearing upon this subject may be quoted. Wadsworth and Wheeler (1928) claim to have obtained an avirulent diphtheroid from the Park No. 8 strain. Six inoculations were made into

Uschinsky's medium, only one of which grew. The diphtheroid isolated from this cultivation was completely avirulent and atoxic, and all attempts to restore its virulence by culture methods in a variety of media and by passage completely failed. But all other attempts to duplicate this experiment with the No. 8 and other strains failed, and the avirulent diphtheroid obtained fermented *saccharose,* which the original strain did not. The more likely explanation of this observation surely is that an adventitious diphtheroid was isolated, and not that Park No. 8 was converted into an avirulent diphtheroid.

C. coryzæ segmentosum (*Cautley, 1896*), *syn.* B. septus (*Benham, 1906*).

Cautley (1894, 1896) recovered from the nasal secretions of a series of cases of 'common cold' a diphtheroid organism consisting of 'short oval bacilli, non-motile, with a pronounced tendency to segmentation of the protoplasm'. It gave a delicate transparent growth on agar, and broth remained clear with a stringy white deposit.

Gordon (1903) recovered what he believed to be this organism from six cases of ordinary colds in the head and he gave the following particulars of the organism : minute colonies on serum showing 'relatively long rods 2 to $2 \cdot 4\mu$ and even $3 \cdot 5\mu$ with very striking beading and segmentation, so that the title *segmentosum* is an apt one'. Neisser's stain, as a rule, showed no metachromatic granules. On glucose broth, slight acidity occurred. On agar (as Cautley found) the bacillus was much shorter and coccoid forms were common. The organism produced no local swelling in guinea-pigs.

Graham-Smith (1906) found the bacilli on serum cultures much as Gordon described them, but on agar noted long well-segmented curved and clubbed forms with very few short elements. Small polar bodies were seen in most specimens from serum, but only in a few from agar. The organism fermented glucose, galactose and lævulose, but not maltose, lactose, dextrin, saccharose, glycerin or mannitol, in the Hiss serum-water medium.

Benham (1906) in 20 out of 21 cases of common cold recovered diphtheroids which he regarded as conforming to the description given by Cautley. Neisser's stain yielded no granules. On sugars he noted that as a rule glucose, lactose, saccharose and maltose were fermented, but saccharose and lactose were liable to give variable results. On agar the appearance was coccoid as in Cautley's original organism, but there is little doubt, from the variable character of the fermentation results, that Benham was dealing with a variety of diphtheroids.

C. xerosis.

This was the first diphtheroid to be recognized, for it was recovered from the eye at least two years before Loeffler isolated *C. diphtheriæ.* It was originally obtained by Kuschbert and Neisser from cases of xerosis conjunctivæ, and was for a time supposed to be causative of that condition, but it was soon found to be present in various forms of conjunctivitis, and a little later was recognized as being frequently present in the normal conjunctival sac (e.g. Lawson, 1899) and in the skin, urethra and other situations (Bergey, 1898). It cannot be stated that all organisms recovered from the eye and grouped as *C. xerosis* form a homogeneous group composed of one species, for other diphtheroids, e.g. *C. hofmanni*, also occur in the eye.

C. xerosis closely resembles *C. diphtheriæ* in morphology and staining reaction. It shows segmentation, metachromatic granules, and involution forms, and retains Gram's stain more strongly than *C. diphtheriæ*. It may show granules with Neisser's stain, but this feature is somewhat inconstant. On blood-serum the growth is fine, delicate, dry and scaly, and is slow in developing, and the colonies are hardly visible in less than 30 hours, and, in later growth, adhere firmly to the medium. Upon agar it will seldom or never grow in primary culture, and in subcultures it forms a thin, translucent, dry film. It will not grow on gelatin in primary culture, and seldom in secondary culture. Broth remains clear with a deposit at the bottom. There is no acid production in milk. In Hiss's serum-water *C. xerosis* produces acid from glucose, maltose, saccharose and mannitol, but not from lactose and dextrin. It is aerobic and forms no pigment. By agglutination tests it is distinct from virulent and avirulent *C. diphtheriæ* and *C. hofmanni*.

C. xerosis by any mode of inoculation is non-pathogenic to guinea-pigs, except for some hyperæmia, œdema and swelling at the site of a subcutaneous injection. This local reaction is not prevented by a preliminary injection of diphtheria antitoxin. No reaction is produced by inoculation into the human conjunctival sac. No toxin is formed, and animals immunized with *C. xerosis* show no immunity to *C. diphtheriæ* and react positively to the Schick test, i.e. no trace of diphtheria antitoxin is formed (Rosenau and Bailey, 1925).

C. acnes.

This organism was observed by Unna (1896) in smears from comedones and was first cultivated by Sabouraud (1897) on an acid glycerin agar. It was cultivated from acne nodules by Gilchrist (1900) and named *Bacillus acnes*. It is regarded as being causative of the comedones of acne, but the suppuration is usually ascribed to secondary infection with *Staphylococcus aureus, &c.* (Whitfield, 1909 and 1912). Gilchrist found that *C. acnes* was agglutinated by the blood of patients with severe acne, and that it was pathogenic for small animals. It was first isolated by anaerobic methods by Hallé and Civatte (1907), deep shake cultures in glucose agar being used.

C. acnes appears in smears made from acne lesions as a short stumpy bacillus, not unlike *C. hofmanni*, often arranged in groups. Under cultivation longer forms develop, and in a week-old culture the rods measure about 1·0μ in breadth by 5μ long, or more, even in thickness but slightly bent; they are arranged singly, in pairs, or in groups. On acid serum-agar coccoid forms predominate under anaerobic conditions, but under aerobic ones long filamentous and branching forms develop. From a glucose agar stab the organisms resemble those in the comedo with some pear-shaped and spindle forms in addition. On potato the rods show segmentation and beading.

C. acnes grows luxuriantly though slowly under microaerophilic conditions, but growth under aerobic conditions is possible if large quantities of material are transplanted, particularly after several subcultures have been made. The best growth is obtained in an acid medium at pH 6·2 to 6·8. Enriching substances like glucose or glycerin are necessary in order to obtain luxuriant cultures. Fleming (1909) employed ordinary nutrient agar with an addition of 1 to 5 per cent. of oleic acid, Südmersen and Thompson (1910) a medium composed of three parts of 3 per cent. nutrient agar, reaction +40, with one part of horse-blood or serum, with anaerobic cultivation in both cases. On ordinary nutrient agar no growth occurs, in a glucose agar stab a dense growth appears, in ordinary broth the broth remains clear with a grey granular deposit. Litmus milk is unchanged.

According to Gilchrist, *C. acnes* is pathogenic to mice and guinea-pigs, but Südmersen and Thompson found it pathogenic to mice only by intraperitoneal inoculation.

Südmersen and Thompson state that at least two, if not more, distinct types of this organism exist and pass as *C. acnes*. The differences concern mainly fermentative activity. Two strains carefully examined by the authors under aerobic and anaerobic conditions, agreed in fermenting glucose, maltose, galactose, inulin, arabinose, glycerin and mannitol, out of a long series of test carbohydrates. One strain, however, fermented saccharose actively, but not raffinose, the other fermented raffinose actively but saccharose only feebly. Neither attacked lactose. Fermentation of the test substances did not occur in any case before the end of the first week of incubation.

Remarkably good clinical results are usually obtained in acne by vaccine treatment. When the tendency to suppuration is marked, an ordinary staphylococcus vaccine (*Staph. aureus*, or *Staph. albus*, or the two mixed) often gives good results, but when comedones are a prominent feature a mixed vaccine of *C. acnes* with staphylococcus may serve better. The treatment may be commenced with 5 to 10 millions of the former, with 100 to 200 millions of the latter, and repeated weekly or thereabouts with gradually increasing doses.

C. typhi exanthematici.

Plotz (1914) recovered this microaerophilic diphtheroid from the blood and organs of typhus fever, but the claim for it to be the essential causative agent has now been practically withdrawn. Organisms apparently indistinguishable from it have been recovered from organs in diseases other than typhus, e.g. from spleens removed at operation.

The fermentative activity of this organism was investigated by Plotz, Olitsky and Baehr (1915) on surface cultures in Buchner tubes. Four strains were examined, three from an epidemic and one from an endemic source, and all four behaved alike in fermenting glucose, maltose, galactose and inulin. They had no action on saccharose, lactose, dextrin, raffinose, mannitol or arabinose. The cultural characters of this organism agree closely with those of *C. acnes.*

C. hodgkini.

Considerable attention has been paid, especially in America, to the occurrence of diphtheroids in obscure conditions accompanied by lymph-gland enlargement. This study was stimulated by certain results obtained by Fraenkel and Much (1910), who recovered from glands in Hodgkin's disease a Gram-positive beaded organism without acid-fastness which they believed to represent an early phase in the life-cycle of an acid-fast organism causally related to Hodgkin's disease.

Bunting and Yates (1913) isolated a diphtheroid from glands of Hodgkin's disease which they named *C. hodgkini.* It is a comparatively large diphtheroid and shows well-marked barring and clubbing. It grows well on Loeffler's serum, Dorset's egg medium, and ordinary agar. The growth is at first glistening and greyish, later becoming more opaque and white. In broth a slimy deposit gradually forms without general turbidity. Bunting and Yates (1915) claimed that inoculated into monkeys it produced a chronic progressive lymphangitis closely resembling Hodgkin's disease, but this has not been confirmed.

Torrey (1916) also examined abnormal lymph glands, including those of Hodgkin's disease, and found different types of diphtheroids present, the same gland on occasion yielding as many as five different types. He isolated a special anaerobic form, *C. lymphophilum*, which may be identical with Bloomfield's (1915) anaerobic diphtheroid.

C. hoagii.

C. hoagii was first recovered by Hoag (1907) under the name of ' Organism X ' from the eye, throat, lungs, empyema cavities, &c. It was named *B. hoagii* by Morse (1912[1]). It is a small diphtheroid, often coccoid, showing involution forms. Granules are commonly present with Neisser's stain. It is easily cultivated and develops rapidly on serum and agar with moist creamy growths, usually grey at first, but often becoming salmon-pink later. On gelatin growth is slow with fine dry yellowish colonies. A faint salmon-pink or salmon-buff growth occurs on potato and a general turbidity is produced in broth, with or without pellicle. According to Hoag, the organism ferments glucose, saccharose and maltose; according to Morse, glucose and saccharose but not maltose or glycerin. It is usually non-pathogenic to guinea-pigs, rabbits, and white rats, though Hoag observed purulent inflammation and death occasionally in guinea-pigs.

C. enzymicum.

C. enzymicum was obtained by Mellon (1916) from the pleural cavity in an obscure case of lung infection, and is probably identical with an organism described by Walker and Adkinson (1917). It was agglutinated by the patient's serum in a dilution of 1 to 80 as against 1 to 15 for normal sera. It is diphtheroid of the barred type on blood-agar, becoming coccoid in certain media. On blood-agar fine transparent colonies develop, becoming later translucent or grey. In glucose broth there is a scanty granular growth without turbidity. Milk becomes acid and later curdled : there is no growth on potato. Glucose, maltose, lactose and dextrin are always, and saccharose, glycerin, mannitol, inulin, salicin and raffinose sometimes, fermented. The organism is virulent to rabbits by intravenous inoculation, and filtrates of broth cultures are also toxic. It possesses considerable resistance to desiccation and antiseptics.

DIPHTHEROIDS AS ÆTIOLOGICAL AGENTS IN DISEASES OF ANIMALS.

Ulcerative lymphangitis of horses and ovine pseudo-tuberculosis. The organisms recovered from these affections in horses and sheep are generally held to be identical (*B. pseudo-tuberculosis ovis*, Preisz and Nocard).

According to Hall and Stone (1916), the diphtheroid grows well on Loeffler's medium and shows slender clubbed forms with metachromatic granules. On broth a pellicle develops as with *C. diphtheriæ*. The sugars fermented were glucose and maltose only, saccharose, dextrin, mannitol and glycerin being unaffected (Hiss's serum-water). Intraperitoneal injection of the organism in male guinea-pigs is followed usually by orchitis as with *B. mallei* or by suppurative processes in omental lymph nodes and spleen. A soluble toxin is formed, which may cause a fatal intoxication of the guinea-pig in a dose of 1 c.cm. of the filtered culture, and there is evidence that the toxic action is, to some extent at least, neutralizable by diphtheria antitoxin. It is of very considerable importance to remember that genuine diphtheria bacilli as well as the organism of Preisz-Nocard may be responsible in horses for similar ulcerative lesions (Minett, 1920 ; Petrie, 1921).

Le Soudier and Verge (1924) isolated two strains of this organism from fowls suffering from avian diphtheria, and compared them with other strains. All hæmolysed and fermented glucose, lævulose, maltose, galactose and saccharose, one fermented glycerin in addition, none fermented lactose, dextrin, inulin, mannitol and dulcitol. Pathogenicity varied, one strain was avirulent to the rabbit, guinea-pig and fowl, others sometimes produced progressive cachexia and death, occasionally small abscesses, in the rabbit and guinea-pig. Barratt's fermentation results are given in Table III, p. 137.

Pseudo-tuberculosis murium. This disease of mice is caused by a diphtheroid *B. pseudo-tuberculosis murium* (Kutscher), which is probably identical with Bongert's organism, *Corynethrix pseudo-tuberculosis murium*, and is pathogenic for mice only. In these animals it gives rise, on intraperitoneal injection, to necrotic actinomycosis-like foci in the viscera and omentum.

Barratt's fermentation results are given in Table III, p. 137.

Corynebacterial pyæmia of foals. A disease occurring in Australia and characterized by pneumonia, pyæmia and internal abscesses. Bull (1924) isolated an aerobic diphtheroid, *C. equi*, which was somewhat Hofmann-like in morphology, and occasionally gave a weak Neisser reaction. It grew luxuriantly on ordinary media, the growths on solid media tending to be yellowish pink in colour. Broth became turbid with a heavy deposit. No change, even after 14 days, was produced in litmus milk, glucose, maltose, lactose, saccharose, raffinose, mannitol, dulcitol, inulin and salicin. The organism was not pathogenic for the rabbit. Inoculated into guinea-pigs, the pus produced little or no effect, but a culture killed after 8 days with an abscess in the spleen. A culture inoculated subcutaneously into the neck of a horse caused a large abscess. A case of this disease, and isolation of the same organism, has also been described by Carne (1927).

REFERENCES.

Note.—References to the Literature published before 1924 will be found in the ' Bibliography ' attached to the Medical Research Council's Monograph, *Diphtheria* (H.M. Stationery Office, 1923). A few omissions from that Bibliography are included in the following list.

ABT, G., 1925, *Ann. Inst. Pasteur*, **39**, 387 ; 1928, *ibid.*, **42**, 1336.
ABT, G. & LOISEAU, G., 1925, *Ann. Inst. Pasteur*, **39**, 114.
ALLISON, V. D. & AYLING, T. H., 1929, *J. Path. Bact.*, **32**, 299.
APFELBACH, C. W., 1925, *J. Infect. Dis.*, **37**, 443.
BAILEY, G. H., 1925, *J. Immunol.*, **10**, 791.
BARRATT, M. M., 1924, *J. Hyg.*, Camb., **23**, 241.
BEHRING, 1890, *Deuts. med. Wschr.*, **16**, 1145 ; 1913, *ibid.*, **39**, 873.
BENGTSON, I. A., 1924, *Bull. U.S. Hyg. Lab.*, No. **136.**
BLAIR, J. E., 1924, *J. Infect. Dis.*, **35**, 401.
VAN BOECKEL, L., 1928, *Ann. Inst. Pasteur*, **42**, 1098.
BÖHME, W. & RIEBOLD, G., 1924, *Münch. med. Wschr.*, **71**, 232.
BRAUN, H. & MÜNDEL, FR., 1927, *Zbl. Bakt.*, Abt. I, Orig., **103**, 182.
BRETONNEAU, P. F., 1826, *Des inflammations spéciales du tissu muqueux et en particulier de la diphthérite* . . . . , Paris, pp. 540.
BROWN, H. C., DUNCAN, J. T. & HENRY, T. A., 1924, *J. Hyg.*, Camb., **23**, 1.
BULL, C. G. & MCKEE, C. M., 1924, *Amer. J. Hyg.*, **4**, 101.
BULL, L. B., 1924, *J. Comp. Path.*, **37**, 294.
CAIGER, F. F. & O'BRIEN, R. A., 1924, *Lancet*, Lond., ii, 53.
CARNE, H. R., 1927, *Veterin. Res. Rep.*, No. **3**, Sc. Bull. No. 29, Dep. of Agricult., N.S.W.
COWAN, M. L., 1927, *Brit. J. Exp. Path.*, **8**, 6.
CROWELL, M. J., 1926, *J. Bact.*, **11**, 65.
DERNBY, K. G., 1926, *J. Gen. Physiol.*, **8**, 311.
DERNBY, K. G. & WALBUM, L. E., 1923, *Biochem. Z.*, **138**, 505.
DREYER, G. & WALKER, E. W. AINLEY, 1909, *J. Path. Bact.*, **14**, 28.
DUDLEY, S. F., 1928, *Brit. J. Exp. Path.*, **9**, 290 ; 1929, *Quart. J. Med.*, **22**, 321.
EHRLICH, P., 1897, *Klin. Jahrb.*, **6**, 299.
FALK, I. S., JENSEN, L. B. & MILLS, J. H., 1928, *J. Bact.*, **15**, 421.
FELIX, A., 1929, *J. Hyg.*, Camb., **28**, 418.
FITZ-GERALD, J. G., 1928, *Ann. Inst. Pasteur*, **42**, 1089.
FLECKSEDER, R., 1916, *Wien. klin. Wschr.*, **29**, 637.

Fleming, A., 1909, *Lancet*, Lond., i, 1035 ; 1910, *Brit. Med. J.*, i, 1382.

Forbes, J. Graham, 1927, *Sp. Rep. Ser. Med. Res. Coun. Lond.*, No. 115 ; 1928, *Proc. R. Soc. Med.*, **21**, 854.

Fujioka, N., 1928, *Z. ImmunForsch.*, **57**, 466.

Gallinek, A., 1928, *Z. Hyg. InfektKr.*, **108**, 522.

Gibbs, C. S. & Rettger, L. F., 1927, *J. Immunol.*, **13**, 323.

Gilchrist, T. C., 1900, *Johns Hopk. Hosp. Rep.*, **9**, 409.

Gins, H. A. & Jermoljewa, Z., 1928, *Z. Hyg. InfektKr.*, **109**, 26.

Glenny, A. T., 1925, *J. Hyg.*, Camb., **24**, 301.

Glenny, A. T. & Hopkins, B. E., 1923, *Brit. J. Exp. Path.*, **4**, 283.

Glenny, A. T. & Pope, C. G., 1927, *J. Path. Bact.*, **30**, 587.

Glenny, A. T. & Waddington, H., 1926, *J. Path. Bact.*, **29**, 118.

Glenny, A. T., Pope, C. G. & Waddington, H., 1928, *J. Path. Bact.*, **31**, 133.

Glenny, A. T., Pope, C. G., Waddington, H. & Wallace, V., 1925, *J. Path. Bact.*, **28**, 463 ; 1926, *J. Path. Bact.*, **29**, 31.

Grasset, E., 1926, *C.R. Soc. Biol.*, Paris, **95**, 1407.

Gundel, M., 1927, *Zbl. Bakt.*, Abt. I, Orig., **101**, 337.

Gundersen, E., 1925, *Norsk. Mag. Laegevidensk.*, **86**, 477.

Gutstein, M., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 243.

Halle, J. & Civatte, A., 1907, *Ann. Derm. Syph.*, Paris, 4 s., **8**, 184.

Harries, E. H. R., MacFarlane, W. M. & Gilhespy, F. B., 1927, *Lancet*, Lond., ii, 646.

Harvey, W. C., 1928^1, *Proc. R. Soc. Med.*, **21**, 850 ; 1928^2, *Lancet*, Lond., ii, 58 ; 1929, *J. Hyg.*, Camb., **28**, 228.

Heinbecker, P. & Irving-Jones, E. I. M., 1928, *J. Immunol.*, **15**, 395.

Hirschfeld, L., 1927, *Z. ImmunForsch.*, **54**, 81.

Hirszfeld, H., Hirszfeld, L. & Brokman, H., 1924, *J. Immunol.*, **9**, 571.

Jensen, L. B. & Falk, I. S., 1928, *J. Bact.*, **15**, 367.

Jensen, L. B., Falk, I. S., Tonney, F. O. & White, J. L., 1928, *J. Bact.*, **15**, 413.

Kahn, D., 1924, *Amer. J. Roentgen*, **12**, 343.

Killian, H., 1924, *Z. Hyg. InfektKr.*, **102**, 262.

Klebs, E., 1883, *Verhandl. d. Congr. f. innere Med.*, Wiesbaden, 139.

Klein, E., 1897, *Rep. Med. Off. Local Gov. Bd.*, 1896–7, Lond., **26**, 267.

Kliewe, H., 1926–7, *Zbl. Bakt.*, Abt. I, Orig., **101**, 6, 44, 199.

Kliewe, H. & Hofmann, A., 1927, *Mschr. Kinderhlk.*, **35**, 318.

Kliewe, H. & Westhues, M., 1925, *Münch. med. Wschr.*, **72**, 587.

Lawson, A., 1899, *Trans. Jenner Inst. Prev. Med.*, 2 s., 56.

Ledingham, J. C. G., 1926, *J. State Med.*, **34**, 2.

Le Soudier & Verge, J., 1924, *Ann. Inst. Pasteur*, **38**, 358.

Levinthal, W., 1926, *Z. Hyg. InfektKr.*, **106**, 679.

Locke, A. & Main, E. R., 1928, *J. Infect. Dis.*, **43**, 41.

Loeffler, F., 1884, *Mitt. GesundhAmt*, **2**, 421–499.

McCartney, J. E. & Harvey, W. C., 1928, *Proc. R. Soc. Med.* (Sect. Epidemiol. & State Med.), **21**, 845.

MacIntyre, D., 1926, *Lancet*, Lond., i, 855.

McSweeney, C. J. & Morgan, W. P., 1928, *Lancet*, Lond., ii, 1201.

Madsen, Th. & Schmidt, S., 1926, *Ann. Inst. Pasteur*, **40**, 300.

Mann, G., 1906, *Chemistry of the Proteids*, London (Macmillan & Co.).

Martin, L., Loiseau, G. & Gidon, V., 1924, *C.R. Soc. Biol.*, Paris, **91**, 332.

Martin, L., Loiseau, G. & Laffaille, A., 1928, *Ann. Inst. Pasteur*, **42**, 1010.

May, P. M. & Dudley, S. F., 1929, *Lancet*, Lond., i, 656.

Metropolitan Asylums Board, 1929, *Ann. Rep.* 1928–9, 240.

Möhrke, W., 1926, *Zbl. Bakt.*, Abt. I, Orig., **100**, 145.

Moloney, P. J. & Fraser, C. J., 1929, *Ann. Inst. Pasteur*, **43**, 129.

Moloney, P. J., Fraser, D. T. & Fraser, C. J., 1929, *Ann. Inst. Pasteur*, **43**, 124.

Neale, A. V., 1928, *Lancet*, Lond., ii, 1342.

O'Brien, R. A., Okell, C. C. & Parish, H. J., 1928, *Publ. Hlth.*, Lond., **41**, 181 ; 1929, *Lancet*, Lond., i, 149.

Oertel, M., 1871, *Deut. Arch. klin. Med.*, **8**, 242–354.

Okell, C. C. & Baxter, E. M., 1924, *J. Path. Bact.*, **27**, 439.

Okell, C. C. & Parish, H. J., 1926^1, *J. Hyg.*, Camb., **25**, 355 ; 1926^2, *ibid.*, **25**, 363.

PARISH, H. J., 1927, *Brit. J. Exp. Path.*, **8**, 162.
PARISH, H. J. & OKELL, C. C., 1926, *Brit. J. Exp. Path.*, **7**, 173; 1928, *Lancet*, Lond., ii, 322.
PARK, W. H., 1913, *Am. J. Obstet.*, **68**, 1213.
PARKER, H. B., 1928, *Brit. J. Exp. Path.*, **9**, 207.
PESCH, K. L., 1924, *Zbl. Bakt.*, Abt. I, Orig. **92**, 27.
PIEPER, E., 1928, *Deuts. med. Wschr.*, **54**, 192.
POVITZKY, O. G., 1929, *J. Immunol.*, **16**, 421.
RAMON, G., 1928, *Ann. Inst. Pasteur*, **42**, 959.
ROSENAU, M. J. & BAILEY, G. H., 1925, *J. Infect. Dis.*, **37**, 97.
ROUX, E. & YERSIN, A., 1888, *Ann. Inst. Pasteur*, **2**, 629; 1889, *ibid.*, **3**, 273; 1890, *ibid.*, **4**, 385.
SABOURAUD, R., 1897, *Ann. Inst. Pasteur*, **11**, 134.
SALECK, W., 1927, *Arch. Hyg.*, Berl., **98**, 32.
SALOMONSEN, C. J. & MADSEN, TH., 1898, *C.R. Acad. Sci.*, Paris, **126**, 1229.
VAN SAUN, A. I., 1923, *J. Infect. Dis.*, **33**, 124.
SCHICK, B., 1908, *Münch. med. Wschr.*, **55**, 504.
SCHMIDT, M. S., 1925, *Ann. Inst. Pasteur*, **39**, 875.
SDRODOWSKI, P. & CHALAPINA, K., 1927, *Zbl. Bakt.*, Abt. I, Orig., **101**, 350.
SMITH, J., 1923, *J. Hyg.*, Camb., **22**, 1.
SMITH, TH., 1907, *J. Med. Res.*, **16**, 359.
STARK, C. N., SHERMAN, J. M. & STARK, P., 1928, *J. Infect. Dis.*, **43**, 569.
STEWART, H. E., 1923, *Amer. J. Electrother.*, **12**, 396.
STONE, R. L., 1923, *J. Infect. Dis.*, **33**, 312.
SÜDMERSEN, H. J. & THOMPSON, E. T., 1909, *J. Path. Bact.*, **14**, 224.
SUGG, J. Y., FLEMING, W. L. & NEILL, J. M., 1927, *J. Exp. Med.*, **46**, 909.
THOMSON, D. & THOMSON, R., 1926, *Ann. Pickett-Thomson Res. Lab.*, **2**, 51.
THOMSON, F. H., MANN, E. & MARRINER, H., 1929, *Metrop. Asylums Bd. Ann. Rep.* 1928–9, 304.
UNNA, P. G., 1896, *Histopathology of the Diseases of the Skin*, trans. by Norman Walker, p. 361 (Edinburgh).
VIRCHOW, R., 1847, *Virchows Arch.*, **1**, 207.
WADSWORTH, A. & WHEELER, M. W., 1928, *J. Infect. Dis.*, **42**, 179.
WALKER, I. C. & ADKINSON, J., 1917, *J. Med. Res.*, **35**, 391.
WALLACE, U., 1927, *J. Path. Bact.*, **30**, 667.
WARTHIN, A. S., 1924, *J. Infect. Dis.*, **35**, 32.
WATSON, A. F. & LANGSTAFF, E., 1926, *Biochem. J.*, **20**, 763; 1927, *J. Path. Bact.*, **30**, 383.
WATSON, A. F. & WALLACE, U., 1924, *J. Path. Bact.*, **27**, 271, 289.
WERNICKE, E., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, 2te Aufl., **5**, 1011.
WHITFIELD, A., 1909, *Lancet*, Lond., i, 1207; 1912, *Brit. Med. J.*, ii, 286.
ZDRODOWSKI, P. & HALIAPINE, C., 1928, *Ann. Inst. Pasteur*, **42**, 1424.

CHAPTER IV. *BACILLUS TUBERCULOSIS.*

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Introduction.

Tuberculosis may be defined as a slow growing granuloma affecting human beings, domesticated and wild animals, due to a member of the genus Mycobacterium (Lehmann and Neumann)—*Mycobacterium tuberculosis*—referred to in this work as *Bacillus tuberculosis*. Varieties of this organism exist which are particularly prevalent in particular genera or groups in the animal kingdom, and are designated by the names of these genera or groups; thus, *B. tuberculosis* human type, bovine type, avian. In this chapter these three varieties are treated together, while a fourth, the bacillus of tuberculosis of cold-blooded animals, is dealt with separately.

History.

By W. Bulloch.

The subject of the causation of tuberculosis is one of great extent, and those who wish to understand the gradual developments of the doctrines held to-day will find them traced in detail in the works of Waldenburg (1869), Johne (1883) and Predöhl (1888). The limits of space prevent any reference to the older views on the cause of consumption, for the idea of tubercle and tuberculosis had not yet emerged. This was first apparent in the writings of Francis Sylvius (De le Boë) (1671), who used the term tubercles (tubercula minora vel majora) to indicate the appearances of the lesions found in the lungs of consumptives. He believed that the tubercles were produced from some invisible glands in the lungs. This idea was contradicted by P. Desault (1733), a physician of Bordeaux, who maintained that tubercles were new productions rather than an enlargement of something pre-existing. His observations were overlooked and no further advance was made until English workers made known the probable nature and development of what every pathologist by that time called ' tubercles '. Among these English workers may be especially named William Stark (1740–70), whose work was published in 1788, and Matthew

Baillie (1793), both pupils of John Hunter at St. George's Hospital. They gave very accurate descriptions of the lesions of consumption and the seed they sowed took root in France at the beginning of the nineteenth century and culminated in the work of A. Portal (1809), G. L. Bayle (1803, 1805, 1810), and particularly of Laennec (1819) and Louis (1825).

G. L. Bayle, himself a consumptive, was the creator of the modern idea of tuberculosis as a morbid process, and his work, not quite complete, was advanced through the masterly observations of his friend Laennec (1781–1826), who also succumbed to the disease. From the fact that in the bodies of consumptives tubercles are found not only in the lungs but also in other parts, Bayle considered that the disease was essentially a dyscrasia or diathesis. He distinguished various types of phthisis, of which the tuberculous was one variety. Laennec, however, was of opinion that tubercle is really a disease and that consumption is tubercle of the lungs. Wherever tubercles occur the disease is tuberculosis and in thus unifying many conditions previously believed to be different, Laennec is justly regarded as a great pioneer in the history of tuberculosis. His description of the appearances and course of the tuberculous process has indeed become classic. His doctrines, revolutionary as they were for the time, became the source of an enormous amount of controversy, but proved in the end to be correct.

Much was done by Virchow in clearing up discrepancies and in directing attention to the pathognomic signs of tubercle, but no great advance took place with regard to the causation of the tuberculous process. Nearly every one, including Virchow (1865), regarded it as a constitutional condition, and, with one or two isolated exceptions, no one believed that tubercle was a communicable disease. Buhl (1857) as the result of an extensive pathological study pointed out that in acute miliary tuberculosis other lesions apparently of greater age can be found in some particular part of the body, and he suggested that these larger lesions stood to miliary tubercles in the relation of cause and effect. He considered that tuberculosis is a specific absorptive disease of infective character and due to the entrance into the blood of a special poison—the tuberculous matter.

The infectivity of tubercle was, however, proved by the French military surgeon, J. A. Villemin (1827–92), in a series of epoch-making papers published between 1865–9, and in a large book (1868). In his first communication to the Academy of Sciences (4th December, 1865) he gave details of inoculations with human tuberculous material in nine rabbits, all of whom showed tuberculous lesions some months later. Six uninoculated rabbits living in the same cages with the inoculated remained healthy, and three rabbits inoculated with material from cholera dejecta, phlegmonous pus or anthrax, showed no changes like those of the animals inoculated with tuberculous matter. Villemin (1866) greatly enlarged his experiments and showed that bovine tubercle is transmissible to rabbits, and human tubercle to guinea-pigs. In dogs, sheep, goats and birds, human tubercle had little effect. Even in his first communication, Villemin (1865) stated his conviction that ' tubercle is a specific affection and its cause is an inoculable agent '. In his opinion tubercle was a disease of the type of syphilis or glanders. It is interesting to recall that Villemin found bovine to be much more virulent for rabbits than human tubercle. In his later work (1868) Villemin showed that the blood of consumptives apparently does not contain the tuberculous virus, which is, however, present in the sputum and can be conveyed to animals by inhalation.

Villemin's work created a great sensation and was repeated in many places with divergent results. Pathologists like Cohnheim and B. Fraenkel (1869), Burdon Sanderson (1867, 1868), Wilson Fox (1868), Waldenburg (1869), were among those who failed to find any evidence of the specificity of inoculated tubercle, but the correctness of Villemin's contention began to be apparent and largely through the famous experiments of Cohnheim and Salomonsen (1878) on the effect of inoculating animals in the anterior chamber of the eye. By this procedure they showed that pure tuberculous material from man causes in rabbits and guinea-pigs tuberculous lesions in the iris, whereas impure tubercle or material not tuberculous at all has an entirely different effect. These results were extended and confirmed especially by Hänsell (1879) and Salomonsen (1879), and from that moment the belief in a specific virus for tubercle became fixed and a search for it began.

The successful finder was Robert Koch (1882), who discovered the organism called by him tubercle bacillus, or for short ' T.B.'. He announced his great discovery to the Physiological Society of Berlin on 24th March, 1882. His first paper was published shortly afterwards (Koch, 1882) and with complete details in 1884. By the application of alkaline methylene blue followed by Bismarck brown he found in sections of tuberculous matter a characteristic bacillus hitherto unknown. Following this clue he showed the same organism in 33 divers tuberculoses of man and animals. The history of his staining method has been clearly given by F. Loeffler (1907) and seems to have been largely the result of an accident. Koch was cautious in his further advance and resorted to cultural methods for isolation of the bacillus. After many failures he succeeded in growing it on ox- or sheep-serum which had been sterilized by heat at 58° C. and finally stiffened at 65° C. On this blood-serum medium he grew the long-sought organism and showed that it was characteristic. The pure cultures, isolated from the lesions of experimental tubercle in guinea-pigs, were inoculated into a large number of animals by a great variety of methods, and in practically all instances reproduced tuberculosis. All his experience with tubercle bacilli led him to the belief that it was not only a concomitant of the disease but its cause, and even in his first communication he emphasized the great importance of inhalation of human tuberculous sputum as the dominating element in the transmission of the disease in man. He supposed (incorrectly) that the persisting virulence of tuberculous material must be due to the presence of spores in the bacillus, and he expressed his view—as had Klebs (1870) and others—that the tubercle bacilli of man and animals are identical. The essential features of Koch's original publication (1882) were: the proof of constant presence of the bacillus, its isolation in pure culture and the production of tubercle by the application of these pure cultures to the bodies of healthy animals. Koch's discovery was at once confirmed all over the world and it stamped him as one of the great investigators of his time.

An improved stain for tubercle bacilli was discovered almost immediately by Ehrlich and communicated by him on 1st May, 1882. This was composed of gentian violet or fuchsine fortified by the presence of aniline dissolved in water. Finding that preparations stained by these solutions could not be decolorized by Bismarck brown as in Koch's method, Ehrlich resorted to the use of strong solutions of nitric acid and discovered the fundamental acid-fast property of the tubercle bacillus. This admirable method of Ehrlich was accepted by Koch as superior to his own, and became, and has remained, the main method for demonstrating tubercle bacilli. It is referred to, not by the name of Ehrlich, but of Ziehl-Neelsen, by which it is known in every laboratory. The only contribution which Ziehl made was the substitution of carbolic acid for aniline, although Ehrlich had also discovered that carbolic acid could be used as a less perfect substitute. It was Neelsen, of Dresden, who recommended the use of carbol fuchsine (instead of Ziehl's carbol-gentian violet) and of sulphuric acid (instead of Ehrlich's nitric acid). Neither Ziehl nor Neelsen published their so-called method. It is to be found only in a footnote in a paper by Johne (1885).

Koch's complete paper, entitled 'Die Aetiologie der Tuberkulose', appeared in 1884 and is a great amplification of what he published in 1882. In this he gave a record of 98 cases of human and 34 of animal tuberculoses. He inoculated with tuberculous matter no fewer than 496 animals, including 273 guinea-pigs and 105 rabbits; and from the experimental tubercle of the former he raised 43 pure strains of T.B., grew them for months, and inoculated them into more than 200 animals of all kinds. Full details were given of all his experiments. He still held to the belief of the unity of tubercle bacilli of man and animals, but reckoned that human bacilli from phthisical sputum dominated the scene so far as concerns human tuberculosis.

The decade following Koch's work created a voluminous literature, and was largely occupied in filling up trivial lacunæ, but everywhere his observations were confirmed in all essential details.

The next outstanding event was Koch's announcement (1890[1]) at the Tenth International Congress of Medicine in Berlin, that he had discovered a certain substance which was said to prevent the successful inoculation of tubercle cultures in animals or even to bring to a standstill a tuberculosis already manifest. The nature of the mysterious 'Heilmittel' was only slowly disclosed in subsequent papers by Koch (1890[2], 1891[1 & 2]), and was at last stated to be a glycerin extract of pure cultures of tubercle bacilli and was called by Koch (1891[2]) 'tuberculin'. An enormous interest was aroused in the medical world by Koch's suggestions that a cure for tuberculosis was attained, but it was not long before the early hopes were shattered by the results obtained by many pathologists and more especially by Virchow (1891), who alleged that the ' Heilmittel ' of Koch often led to disastrous consequences for the patient. The inner history of Koch's tuberculin is not and possibly will never be accurately known. There is reason to believe that his hand was forced before he had completed his work. Koch evidently had faith in bacillary remedies against tubercle, for he continued his work and produced a new tuberculin ' T.R. ' in 1897, and also made other attempts to retrieve the position. An extraordinary amount was written between 1890 to 1900 on every aspect of the tuberculin question. In 1901, in a lecture in St. James's Hall at the British Congress of Tuberculosis, Koch created a new sensation by his assertion that bovine tubercle bacilli and human tubercle were not identical, and related the results of experiments with his co-worker Schütz to the effect that human tubercle bacilli do not cause a progressive destructive tuberculosis in cattle. With regard to the other side of the question, he considered that if bovine bacilli were pathogenic for man at all it must be a relatively rare event, and was of such trivial importance that it did not indicate the necessity for the application of definite measures. Koch's view on the relation of human and bovine bacilli was not altogether new ; for although it had been assumed that the two types of bacilli were identical, there was a good deal of evidence from the time of Villemin onwards that there were at least differences in the degree of infectivity, especially in the effect on rabbits and cattle. Bacteriological differences had also been pointed out by Theob. Smith (1896, 1898) and others. All the same Koch's statements in London came as a bombshell, disturbing cherished beliefs, and arousing intense criticism. Investigations were commenced in all countries, either by individuals or by specially appointed commissions, and entailed years of labour and great cost. In the main, it may be said that Koch's conclusions have proved to be correct ; and it is admitted to-day that pulmonary consumption of man, which constitutes the great mass of human tuberculous conditions, is to all intents and purposes transmitted by man. In later years there have been refinements in all branches of tuberculosis, diagnostic and therapeutic, but we still lack a really effective treatment for consumption. It may be mentioned that to-day no fewer than 30 journals are devoted to the special study of tuberculosis in the different countries.

REFERENCES.

BAILLIE, M., 1793, *The morbid anatomy of some of the most important parts of the human body*, London.

BAYLE, G. L., 1803, *J. méd., chir., pharm., etc.*, **6**, 3 ; 1805, *ibid.*, **9**, 427 ; 1810, *Recherches sur la phthisie pulmonaire*, Paris.

BUHL, 1857, *Z. rat. med.*, n.F., **8**, 48.

COHNHEIM, J. & FRAENKEL, B., 1869, *Virchows Arch.*, **45**, 216.

COHNHEIM & SALOMONSEN, 1878, *Jber. schles. Ges. vaterl. Kult.*, **55**, 222.

DESAULT, P., 1733, *Dissert. sur le mal. vénériens*, etc., Bordeaux.

EHRLICH, 1882, *Deuts. med. Wschr.*, **8,** 269.

FOX, WILSON, 1868, *Brit. Med. J.*, i, 499, 525, 551.

HÄNSELL, 1879, *Ber. ophthal. Ges.*, **12,** 193.

JOHNE, A., 1883, *Die Geschichte der Tuberkulose* . . . . , Leipzig; 1885, *Fortschr. Med.*, **3,** 198 (footnote p. 200).

KLEBS, 1870, *Virchows Arch.*, **49,** 291.

KOCH, R., 1882, *Berl. klin. Wschr.*, **19,** 221 ; 1884, *Mitt. GesundhAmt.*, **2,** 1–88, 10 pl. ; 1890^1, *Zbl. Bakt.*, **8,** 563 ; 1890^2, *Deuts. med. Wschr.*, **16,** 1029 ; 1891^1, *ibid.*, **17,** 101 ; 1891^2, *ibid.*, **17,** 1189 ; 1897, *ibid.*, **23,** 209 ; 1901, *ibid.*, **28,** 549.

LAENNEC, R. T. H., 1819, *De l'auscultation médiate* . . . . , Paris, Tome I, Chap. 2.

LOEFFLER, F., 1907, *Deuts. med. Wschr.*, **33,** 449, 489.

LOUIS, P. C. A., 1825, *Recherches anatomico-pathologiques sur la phthisie*, Par. pp. 560.

PORTAL, A., 1809, *Observations sur la nature et le traitement de la phthisie pulmonaire*, Paris, 2 vols.

PREDÖHL, A., 1888, *Die Geschichte der Tuberkulose*, Hamb. and Leipz., pp. 502.

SALOMONSEN, C. J., 1879, *Nord. med. Ark.*, **11,** No. 12, 1 ; No. 19, 1.

SANDERSON, J. B., 1868, *Rep. Med. Off. Privy Council* (1867), Lond., 1868, **10,** 111 ; also 1869, **11,** 91.

SMITH, THEOB., 1896, *Trans. Ass. Amer. Phys.*, **11,** 75 ; 1898, *J. Exp. Med.*, **3,** 451.

STARK, W., 1788, *The works of the late* . . . . , Lond., 1788, pp. 190.

SYLVIUS (DE LA BOË), F., 1671, *Praxeos medicaæ idea nova*, Lugd. Bat., Lib. I.

VILLEMIN, J. A., 1865^1, *C.R. Acad. Sci.*, Paris, **61,** 1012 (4 Dec.) ; 1865^2, *Gaz. hebd. Méd. Chir.*, 2 s., **2,** 795 ; 1866, *ibid.*, 2 s., **3,** 758, discussion ; 1867, *ibid.*, **4,** 531 ; 1868^1, *ibid.*, 2 s., **5,** 526, 596 ; 1868^2, *Études sur la tuberculose, preuves rationelles et expérimentales de sa spécificité et de son inoculabilité*, Paris, pp. 640.

VIRCHOW, R., 1865, *Virchows Arch.*, **34,** 11 ; 1891, *Berl. klin. Wschr.*, **28,** 49.

WALDENBURG, L., 1869, *Die Tuberculose, die Lungenschwindsucht und Scrofulose*, Berlin, pp. 560.

Morphology of the Tubercle Bacillus.

BY A. STANLEY GRIFFITH.

Tubercle bacilli are slender non-motile organisms which vary in length from 0·5 to 8·0μ, the usual limits being 1·2 to 3·5 or 4·0μ, and in breadth from 0·3 to 0·5μ. In the tissues they are rarely perfectly straight, but are generally slightly curved and not infrequently crooked or bent at an angle. According to Eastwood (1907), who made a great number of measurements, the average length in 96 preparations varied between 1·23 and 4·12μ, the smallest form measuring 0·5μ, the longest 8μ. The bacilli do not usually stain uniformly, but show clear spaces which when regularly disposed give the stained portions the appearance of a chain of cocci. Sometimes deeply stained globular or oval bodies are seen at the poles or in the length of the bacilli. Both the clear spaces and the darkly stained bodies have been regarded as spores, but it is now generally believed that the former are due to segmentation of the chromophil substance and that the latter are chromatin granules such as are seen in other species of bacteria. The tubercle bacillus reproduces itself not by spore formation but by elongation and transverse fission.

In tuberculous tissues and exudates the number of bacilli is very variable. They are generally numerous in early, acute or progressive lesions and often cannot be demonstrated microscopically in chronic calcareous or fibroid lesions. The bacilli occur singly, in small groups where they lie parallel or at an acute angle to each other, in large groups, or in masses so compact that the outlines of individual organisms are not distinguishable ; rarely one sees a short chain of bacilli. They may be present within intact or degenerating cells, but in human and most animal tissues they are more usually lying free. In the tissues of acutely infected animals, avian bacilli often occur in compact clumps or in loose aggregations having a rosette formation and are generally shorter than mammalian bacilli. In chronic tuberculous processes avian bacilli are indistinguishable from mammalian bacilli.

In cultures from serum mammalian bacilli are very uniform and are usually straight and uniformly stained. Eastwood states that serum grown bacilli vary in length from 0·5 to 2·5μ, the average being about 1·0μ ; organisms measuring 1·5 to 2·5μ are relatively few. Impression preparations of young primary colonies on serum show a characteristic parallel arrangement of the bacilli in long wavy coils, thicker in the middle than at the extremities. When growing freely on glycerin media the bacilli tend to grow longer, the average length being 2·0 to 3·75μ, and they are often curved and stained irregularly, showing characteristic beading.

Avian tubercle bacilli in culture are more pleomorphic than mammalian bacilli. In serum and young egg cultures of avian bacilli, coccus and short oval forms often predominate. In older cultures on egg certain colonies show great variation in morphology and a characteristic feature is the number of forms which are not acid-fast. In a film counter-stained with methylene blue forms resembling chains of streptococci, staining either all red or all blue or partly red and partly blue, may be seen. Club-shaped bacilli and branched forms are frequent.

STAINING METHODS.

Tubercle bacilli possess a peculiarity of chemical or physical constitution which hinders the penetration into their bodies of ordinary aqueous solutions of aniline dyes, while, when once staining has been achieved, they are resistant to the decolorizing action of strong acid.

The Ziehl-Neelsen method of staining (vide Vol. IX) is to be preferred to other methods for its simplicity, trustworthiness and the contrast it gives between the tubercle bacilli stained red and the other elements stained blue.

The tubercle bacillus can also be stained by Gram's method. Stained in this way the bacilli are often very granular, sometimes resembling a short chain of small cocci. For general use this method cannot be recommended.

Much's granules. It often happens that ordinary microscopical examination of a smear preparation of chronic tuberculous lesions stained by Ziehl-Neelsen fails to reveal acid-fast organisms, although that material is infective, as shown by its ability to produce tuberculosis in guinea-pigs. By employing a modification of Gram's method, Much (1908) saw in such material granular rods and granules, sometimes isolated, sometimes grouped together, which, he believed, represented a virulent non-acid-fast form of the tubercle bacillus. The existence of such a form is, however, very doubtful and is not supported by the observations of Bittrolf and Momose (1913), who were unable to demonstrate by Much's method any other form than that stainable by the Ziehl method. In the author's experience acid-fast bacilli can generally be found, if the search is sufficiently prolonged, in tuberculous tissues (for example, cold abscesses); which according to Much ought not to contain them, and direct cultures from such material always yield colonies proportionate in number to the scanty acid-fast elements seen microscopically.

VARIATIONS.

In the tissues tubercle bacilli retain their bacillary form. In cultures, especially old potato cultures, long or thread forms are sometimes seen. They stain with varying degrees of intensity and generally show chromatin granules and sometimes bulbous or club-shaped thickenings. Branched forms are rarely seen in mammalian cultures. They are more common in avian cultures and like the thread forms stain with different degrees of intensity. The branching frequently emanates from a darkly stained spherical point which in some instances is of a greater diameter than the breadth of the bacillus (Eastwood, 1911). The extremities of the branches are often swollen and club-shaped. The occurrence of these forms has brought the suggestion that the tubercle bacillus may be related to the streptothrices. Many regard them as involution or degeneration forms.

DIFFERENCES IN MORPHOLOGICAL CHARACTERS BETWEEN HUMAN, BOVINE AND AVIAN TYPES OF TUBERCLE BACILLI.

Theobald Smith (1898) stated that human tubercle bacilli are on the whole longer and more slender than bovine bacilli and tend more frequently to assume curved uniformly stained or beaded forms. Other observers have found that while these differences obtain as a rule, they are not constant. The microscopic characters of mammalian tubercle bacilli of either type are very variable and it is often impossible on morphological grounds to decide whether the bacilli are of human or bovine origin.

Avian tubercle bacilli are more pleomorphic than mammalian bacilli and in cultures (on egg chiefly) frequently assume branched and club-shaped forms. True branching is rarely seen in mammalian cultures.

THE QUESTION OF A FILTERABLE FORM OF THE TUBERCLE BACILLUS.

Various observers claim to have proved by experiment the existence of a form of the tubercle bacillus which is capable of passing through Berkefeld or Chamberland filters and of producing in susceptible animals an atypical form of tuberculosis. The first suggestion that such a filterable form might exist came from Fontès (1910) who, in the course of work on

Much's granules, made the observation that Berkefeld filtrates of tuberculous pus can give rise in the guinea-pig to enlargement of lymphatic glands and spleen. Microscopically these organs showed Gram-fast granules in the lymphocytes but no acid-fast rods. An emulsion of the spleen of the first guinea-pig inoculated into a second guinea-pig, however, produced glandular enlargement and lesions in the lungs, not typical of tuberculosis, in which acid-fast bacilli were found. Fontès concluded that Much's granules could pass through a Berkefeld filter and on inoculation give rise to atypical tuberculosis in guinea-pigs. Later he expressed the opinion that the granules represented a stage of development of the tubercle bacillus.

In 1921, Vaudremer reported that tubercle bacilli cultivated in a special potato broth produce non-acid-fast filaments containing minute granules which are capable of passing through the pores of a Chamberland L 3 candle ; he also stated that he had obtained colonies of acid-fast bacilli upon Petroff's egg medium sown with the filtrate.

Hauduroy (1923) in collaboration with Vaudremer, cultivated the same filamentous granular forms in 2 per cent. glycerin peptone-water from filtrates of ordinary potato cultures. These filtrate cultures, which contained no acid-fast forms, produced in guinea-pigs inoculated subcutaneously transient local indurations and after intravenous inoculation general enlargement of the lymphatic glands, with caseation occasionally, the caseo-purulent substance yielding cultures which after a few subcultures became acid-fast but did not produce tuberculosis in guinea-pigs. Calmette (1928) was unable to confirm these observations of Vaudremer. Valtis (1924), on the other hand, under the direction of Calmette repeated the original experiments of Fontès and obtained results which appeared to indicate that the tubercle bacillus might exist in ultramicroscopic form capable of passing through the pores of a Berkefeld N or a Chamberland L 2 filter bougie. The filtrates did not yield cultures, but when inoculated into guinea-pigs caused enlargement of the lymphatic glands—particularly the tracheo-bronchial—in which acid-fast bacilli could always be found if the search was sufficiently prolonged. A lesion at the seat of inoculation of the filtrate was never produced. In about 1·0 per cent. of the guinea-pigs nodular lesions rich in bacilli occurred in the organs. Results similar to those of Valtis and Calmette have been obtained by Arloing and Dufourt (1925) and others in France and by many investigators in other countries.

Other investigators, Dessy (1926) and Montemartini (1925) in Italy, Fessler (1926) and Rabinowitsch-Kempner (1927) in Germany, Cooper and Petroff (1928) in America, Alston (1928), Gloyne, Glover and Griffith (1929) in Britain, have obtained only negative results. Calmette thinks that these investigators considered their experiments to have been negative either because they expected to find classical tuberculous lesions in the guinea-pigs, or because their search for acid-fast bacilli in the glands was not thorough enough. Most of the investigators, however, who failed to demonstrate the existence of a filterable form of the tubercle bacillus did not observe glandular enlargement in the guinea-pigs inoculated with filtrates, and, with the exception of Cooper and Petroff, did not find acid-fast bacilli in the glands after prolonged search. Cooper and Petroff found acid-fast organisms in 36 per cent. of the guinea-pigs which had received filtrates, but they also found similar organisms in 33 per cent. of apparently normal guinea-pigs which had not received filtrates. The nature and source of these organisms were not determined. Calmette thinks that the peculiar form of disease—glandular hyperplasia with now and then caseous or nodular changes in one organ or gland—produced by filtrates may be explained on the assumption that the filterable form of the tubercle bacillus has an attenuated virulence. In support of this view he quotes the experiments of Valtis, Nègre and Boquet (1927) and others, who believed that they had succeeded in increasing the virulence of the filtered virus and at the same time converting it into the typical form by passage through the guinea-pig.

Calmette, Valtis and Lacomme (1926) claim also to have proved that the ultramicroscopic form of the tubercle bacillus can pass through the placenta of a pregnant animal and affect the fœtus. These results also have not been confirmed by other investigators.

Calmette (1928) considers that the experimental evidence which has been adduced by himself and his collaborators proves conclusively the existence of a filterable virus in tuberculosis, but it is difficult to understand why, if such a virus exists, so many investigators who have made similar experiments have had entirely negative results.

REFERENCES.

Alston, J. M., 1928, *Edinb. Med. J.*, **35,** 116.

Arloing, F. & Dufourt, A., 1925, *C.R. Acad. Sci.*, Paris, **181,** 826; 1927, *Pr. méd.*, **35,** 17.

Bittrolf, R. & Momose, K., 1913, *Veröff. Koch Stift. Tuberk.*, **4,** 18.

Calmette, A., 1928, *Bull. Inst. Pasteur,* **26,** 889.

Calmette, A., Valtis, J. & Lacomme, M., 1926, *C.R. Acad. Sci.*, Paris, **183,** 835.

Cooper, F. B. & Petroff, S. A., 1928, *J. Infect. Dis.*, **43,** 200.

Dessy, G., 1926, *Boll. Ist. sieroter. Milano,* **5,** 41; cited from *Zbl. Bakt.*, Abt. I, Ref., **84,** 157.

Eastwood, A., 1907, *Royal Commission on Tuberculosis,* 2nd Interim Rep. Appendix, **4,** 255; *ibid,* 1911, Final Rep. Appendix, **5,** 344.

Fessler, A., 1926, *Zbl. Bakt.*, Abt. I, Orig., **98,** 148.

Fontès, A., 1910, *Mem. Inst. Oswaldo Cruz.*, **2,** 181; 1926, *Zbl. Bakt.*, Abt. I, Ref., **83,** 476.

Gloyne, S. R., Glover, R. E. & Griffith, A. S., 1929, *J. Path. Bact.* **32,** 775.

Hauduroy, P. & Vaudremer, A., 1923, *C.R. Soc. Biol.*, Paris, **89,** 1276.

Montemartini, G., 1925, *Boll. Ist. Sieroter. Milano,* **4,** 1.

Much, H., 1908, *Berl. klin. Wschr.*, **45,** 691.

Rabinowitsch-Kempner, L., 1927, *Deuts. med. Wschr.*, **53,** 1982.

Smith, Th., 1898, *J. Exp. Med.*, **3,** 470.

Valtis, J., 1924, *C.R. Soc. Biol.*, Paris, **90,** 19, 74 & 1130; 1926, *ibid.*, **94,** 237 & 376; 1928, *Pr. méd.*, **36,** 113.

Valtis, J., Nègre, L., Boquet, A. & Certonciny, A., 1927, *C.R. Soc. Biol.*, Paris, **97,** 1667.

Vaudremer, A., 1921, *C.R. Soc. Biol.*, Paris, **85,** 10 and 55; 1923, *ibid.*, **89,** 80.

Cultivation of the Tubercle Bacillus.

By A. Stanley Griffith.

The tubercle bacillus is aerobic and grows at temperatures ranging from 29 to 42° C., the optimum being 37 to 38° C. for mammalian bacilli and 40° C. for avian; it requires for initial cultures from tuberculous tissue solid media containing albuminous substances or glycerin. The media should have a neutral reaction (optimum pH 7·0 to 7·6) and a dry surface; a soft moist surface is unfavourable, but a little moisture of condensation at the foot of the tubes is advantageous. Growth is slow and colonies are usually not visible to the naked eye until the eighth or tenth day and sometimes later. The medium must, therefore, be prevented from drying. The presence of glycerin favours the growth of human, avian and some bovine strains, but is not essential. The discovery of its value for the cultivation of tubercle bacilli was made by Nocard and Roux (1887), who added it in the first instance for its hygroscopic properties.

MEDIA.

Media for primary cultures. Cultures of tubercle bacilli can be obtained direct from tuberculous material with certainty only on an egg or a good serum medium. Koch (1884) obtained his first culture on coagulated bovine-serum. Theobald Smith (1905) recommended the serum of the dog. M. Dorset (1902) used a medium prepared from hens' eggs. A. S. and F. Griffith (1907) experimented with a variety of media and they found that an egg medium (see Vol. IX) was the most successful and could always be relied upon for the production of primary cultures; the yolk alone and the mixture of yolk and white were far superior to the white alone. (The various modifications of the egg medium which have been introduced by different authors have no superiority over plain egg as a stock medium for the isolation of tubercle bacilli from tissues.) The sera of a large number of different species of animals were tested and at least one serum of each species gave a high percentage of successes. The serum of the cat was the most uniformly good, while those of the calf and the horse were the least favourable. The serum of the adult ox is superior to that of the calf both for primary cultures and for subcultures. The unfavourableness of some sera seems to be due to a film of salts which forms on the surface. Serum agar is a satisfactory and economical substitute for pure serum. Glycerinated potato will yield primary cultures, though it is not so reliable as egg, while glycerin agar is definitely unfavourable.

Media for subcultures. The tubercle bacillus having once started to grow in culture can be readily subcultivated on a variety of artificial media. The best medium for obtaining growth suitable for cultural and virulence tests is undoubtedly the coagulated serum of the adult bovine; such growth is readily emulsified and easily spread. To ensure that the serum is sterile the blood should be withdrawn by cannula from the jugular vein of the living animal or the carotid artery of the slaughtered animal. It should be heated in the inspissator to 80 or 85° C. only for such length of time as is necessary to coagulate it firmly. The egg medium is unsatisfactory for early subcultures, but is much more suitable for the conservation of cultures than serum media.

For obtaining mass cultures of tubercle bacilli on broth or potato, glucose (2 per cent.) may with advantage be substituted for glycerin. Saccharose and alcohols allied to glycerin are attacked but feebly and are not useful ingredients of a medium. The mineral substances which appear to be essential for the growth of the tubercle bacillus are potassium, magnesium and phosphorus. Iron in trace has a distinctly favourable influence and, according to Frouin and Guillaumie (1923), in the form of the ammoniated citrate greatly increases the power of the tubercle bacillus to utilize glycerin. A large number of synthetic media containing no albumin have been used in analytical work and in the preparation of tuberculin.

Novy and Soule (1925) have shown that for luxuriant growth (human) tubercle bacilli require plenty of oxygen. In order to obtain a rich growth on a single tube of glycerin agar from 100 to 150 c.cm. O_2, roughly corresponding to 500 to 700 c.cm. ordinary air, must be provided. The optimum oxygen pressure was between 40 and 50 per cent.

VIABILITY OF CULTURES.

The length of life of cultures varies with the medium, the strain and the luxuriance of growth. Cultures retain their vitality longer on the egg medium than on serum or other media. It is generally believed that avian strains can survive longer than bovine and bovine than human strains. F. Griffith (1911) made a number of comparisons and found little difference between the various types; the most advanced age at which a culture was able to grow was in the case of the avian, 1,067 days (room temperature), the bovine, 990 days (37·5° C.), and the human, 936 days (room temperature). A. S. Griffith found avian bacilli still alive in a culture 3 years old and human bacilli in a culture 2¼ years old, both kept in the incubator at 37·5° C. On the other hand, cultures of all three types of bacilli may die within a comparatively brief period—from 90 to 180 days.

Primary cultures, especially when the colonies are discrete, remain alive longer than luxuriant subcultures, probably because essential substances in the medium are used up less quickly in the former than in the latter instances. The results show that in order to keep a culture alive for the maximum period the egg medium should be used and sowed sparsely for the purpose of producing discrete colonies.

METHOD OF OBTAINING CULTURES.

Cultures of tubercle bacilli may be obtained directly from any tuberculous tissue or fluid when there are no other organisms present which might overgrow them. Softened caseous substance, pus or other fluids which have been withdrawn aseptically can be spread at once over the surface of the medium. With tissues that cannot readily be broken up the following procedure is recommended. The piece of tissue is put in a short, stout test-tube and broken up with a glass rod ground at the extremity; the fine fragments are then suspended in a few drops of physiological salt solution. This method of emulsification has several advantages over that of placing pieces of tuberculous tissue directly on to the medium. For example, a small tuberculous focus which by the latter method could be used for one tube only, can by the former be used to inoculate a series of tubes, and the liberation of the tubercle bacilli from the tissues increases the chances of securing a culture. There is a further advantage that the emulsion so prepared can be examined microscopically before sowing the tubes, and if contaminating organisms are present recourse may be had to one or other of the following procedures:

1. The emulsion may be treated with a substance which kills the contaminating organisms more quickly than the tubercle bacilli. The substances which have been used for this purpose are formalin, ericolin, antiformin and sodium or potassium hydrate. Of these, antiformin and caustic potash or soda, which have the property of dissolving albuminous material, are to be preferred. Antiformin was introduced by Uhlenhuth (1909) for the purpose of enrichment—through homogenization, liquefaction and centrifugation—of material for cultures and microscopical examination.

The method recommended by Uhlenhuth for obtaining cultures of tubercle bacilli from sputum was as follows: Diluted sputum and antiformin (15 per cent.) are mixed together, thoroughly shaken and allowed to stand for an hour; the liquid is then centrifuged and the supernatant fluid poured off; before use the sediment is washed twice with sterile water. A. S. Griffith (1916) recommends a much simpler procedure which dispenses with the centrifuge. Equal small quantities of sputum (0·5 to 1·0 c.cm.) and dilute antiformin, the percentage varying from 10 to 30 according to the consistency of the sputum, are mixed together. As soon as the mixture becomes clear and homogeneous a sowing is made, and thereafter at intervals, long or short according to the rapidity of solution. The mixture need not be neutralized, and so long as it remains mucinous, will yield cultures even up to three or more days. With percentages of antiformin which cause rapid liquefaction of the medium, the time available during which cultures of tubercle bacilli are likely to be successful may be very brief. In 13 experiments of the writer with thin sputum mixed with 10 per cent. antiformin (5 per cent. in the mixture) solution of the sputum was rapid and the mixtures which yielded pure cultures of tubercle bacilli after 10 minutes' exposure were sterile after further periods of 5 and 10 minutes. Antiformin, therefore, is a reagent which must be used with caution. The secret of success in this method is to sow the culture tubes while the mixture is still mucinous.

Potassium hydrate in 5 per cent. solution may with advantage be substituted for antiformin.

The antiformin and potassium hydrate methods are applicable to the isolation of tubercle bacilli from contaminated tuberculous tissues, pus from lung cavities or open abscesses, fæcal matter and urine. Urine should be first centrifuged and the deposit treated. It is a good plan when attempting cultures from fæces, or centrifuged deposits which contain little albuminous matter, to mix with them a small amount of sterile serum before adding the antiformin or alkali.

Petroff's method of cultivation on a medium containing gentian violet which inhibits the growth of other organisms is uncertain and has no advantages over the antiformin or caustic potash method.

2. The contaminated material may be inoculated into a guinea-pig. This is the most reliable method of obtaining a culture of tubercle bacilli from suspected material when that material is small in amount or grossly contaminated. In the latter circumstance it is advisable to treat the emulsion for an hour with 2·5 per cent. potassium hydrate before inoculation. The tuberculous organs of the guinea-pig most convenient for cultivation experiments are the spleen or caseous lymphatic glands. Where the material inoculated is known to contain acid-fast bacilli, cultures should be attempted from the lymphatic glands adjacent to the seat of inoculation, even in the absence of visible lesions.

CHARACTERS OF GROWTH ON VARIOUS MEDIA.

The bovine type. In primary culture on egg, colonies of the bovine bacillus 2 to 3 weeks old are small, flat and transparent, with slightly raised centres. When very numerous the colonies run together and form a thin moist layer which later becomes translucent. Egg cultures after a few weeks' incubation often show an outcrop of pearly-white secondary colonies. When colonies are few in number they grow large and may become heaped up, creamy irregular nodules. On serum the colonies are flat, dull and scale-like. On glycerin egg the result varies with the strain. Generally the growth of the bovine bacillus is inhibited by glycerin and only few bacilli succeed in forming colonies. With a relatively few bovine strains, colonies may be as numerous on glycerin egg as on plain egg and may grow to a larger size; they, however, remain translucent and moist.

In subculture on serum and egg recently isolated cultures produce thin grey glazed layers. On glycerin media bovine bacilli vary in their manner of growth. Some grow sparsely, others with relative luxuriance, while others again exhibit intermediate grades of luxuriance. A. Eastwood and A. S. and F. Griffith divided bovine strains into three arbitrary grades or classes according to their capacity for growth on glycerin media. Class I contains the most 'dysgonic' (growing with difficulty) strains, the growth of which is hindered by glycerin and does not become visible on glycerin egg and glycerin serum until the second week, generally the tenth day, when discrete colonies appear; there is the same initial delay on glycerin-agar and potato, and on broth the pellicle produced is thin, grey and often broken up into islands. Class II strains show no initial delay on glycerin-serum and produce layers which are similar to, or sometimes rather thicker than those on serum alone. Class III strains grow well on all the glycerin media, forming on glycerin-serum a layer three to four times the thickness of that on serum, on glycerin-agar and potato greyish-white layers of medium thickness, and on glycerin broth moderately thick grey-white pellicles which soon become moist and sink.

The human type. On egg colonies of human bacilli are whitish centrally and more raised than bovine colonies, and on incubation become dry and adherent. The central part of a colony often grows out to form an opaque creamy-white warty mass. On glycerin egg colonies are as numerous as on egg, but grow larger and become definitely creamy. When very numerous they coalesce and form a thick dry granular and sometimes wrinkled layer.

In subculture on egg the human bacillus in early generations forms dull grey uniform layers ; in later generations the growth is greyish-white, dry and tends to adhere to the medium. On bovine serum when this is of a golden yellow colour, human cultures exhibit a yellow pigment, varying from cream to deep yellow. On glycerin media the human bacillus is ' eugonic ', that is, grows readily and luxuriantly, producing thick creamy, wrinkled or warty layers. Old cultures on potato are often deeply pigmented, orange-red or reddish-brown. The surface of glycerin broth quickly becomes covered with a greyish-white membrane, which climbs up the sides of the flask, then thickens, becomes coarsely wrinkled and in places warty and shows no tendency to sink. Broth cultures of mammalian tubercle bacilli have a characteristic pleasant aroma, recalling the scent of flowers.

The avian type. The media which are most favourable for the growth of the avian tubercle bacillus in primary culture are plain egg and glycerin egg. On plain egg the colonies become visible to the naked eye on the ninth to the twelfth day as minute points which under magnification are seen to be transparent droplets. When the colonies are very numerous they may remain transparent and filmy ; more commonly in the third or fourth week pearly-white raised colonies begin to appear ; on further incubation these colonies increase in size and number and become opaque-white and finally creamy-yellow ; they may be rounded and smooth, irregularly nodular or characteristically umbilicated. On glycerin egg the colonies are visible a little sooner than on egg and they grow to a larger size, becoming raised, translucent, then hemispherical, smooth, shiny white, creamy-yellow and finally brownish-yellow. When the colonies are very numerous on glycerin egg they may coalesce to form a thick, moist, creamy-white layer. Fragments of avian tuberculous tissues planted on the surface of egg frequently become covered with a close outcrop of colonies which grow out from the tissue.

On bovine-serum, glycerin-serum, serum-agar and serum glycerin-agar, which are all useful for obtaining primary cultures, the colonies appear at first as minute transparent droplets with an amber tint by transmitted light. Glycerin-agar is unfavourable for the growth of the avian tubercle bacillus in primary culture. On glycerinated potato growth occurs often, though slowly, in the form of minute moist whitish colonies.

Subcultures. The avian bacillus can readily be subcultivated and grows best on media containing glycerin. On plain egg and bovine serum thin, moist, shiny layers are produced which only rarely become thick and opaque. On glycerin-serum and glycerin-egg the growth at first resembles that on serum or egg but continues for a longer time ; the layer becomes translucent, then slightly opaque and finally after several weeks thick, moist, creamy or yellowish ; warty or nodular colonies may grow out. No pellicle forms on the condensation water, but the bacilli usually grow in small masses at the bottom. On glycerin-agar growth is slow but continuous. At first there is a thin, moist, shiny, translucent layer which gradually thickens, becoming greyish-white and finally creamy. The surface of the growth may be smooth or thrown into wrinkles and folds, and become studded with raised secondary colonies. The wrinkled layers have a close superficial resemblance to those produced by the eugonic human bacillus, but the cultures of avian tubercle bacilli are translucent at the margins when viewed by transmitted light and when touched with a platinum wire are characteristically moist and slimy. Sometimes in old cultures there is a formation of reddish pigment, which is irregularly distributed on the tops of the folds ; outcropping colonies may be quite pink. On glycerinated potato the avian tubercle bacillus generally produces an abundant growth. At first greyish-white, it gradually thickens and becomes yellow. The surface may wrinkle or become studded with small colonies, producing a warty layer very similar in appearance to the growth sometimes produced by eugonic human tubercle bacilli. On glycerin broth it grows abundantly though some difficulty is experienced in inducing it to form a surface pellicle. The early growth is extremely thin and scarcely visible. Gradually it thickens, parts becoming opaque greyish-white, others remaining translucent. There is a tendency for bacilli or fragments of growth to fall to the bottom of the flask, where they continue to grow and form a flocculent deposit, the broth remaining clear.

Atypical strains. The great majority of the strains obtained from human beings and the domesticated animals have exhibited the typical cultural characteristics of one or other of the three types of tubercle bacilli. The remainder have not corresponded exactly in their cultural features to any of the recognized types of bacilli. They, however, possessed the virulence of either human or bovine or avian bacilli and are regarded respectively as varieties of these types :

1. Cultural variants of the human type have been obtained from a great variety of human tuberculous lesions and have constituted about 3 per cent. of the total strains of human type. One strain of this type has been obtained from a monkey. The characteristics of these strains are that they grow well on bovine serum and glycerin-serum, forming a yellow pigment like the eugonic human bacillus, while on glycerin-agar, potato and broth

they are dysgonic ; on the latter media they resemble but are not identical with dysgonic bovine strains. The peculiar cultural characteristics are not altered by passage through animals or by prolonged subcultivation on media without glycerin, but some of the strains have manifested instability when grown on glycerin media, acquiring the typical luxuriance of the eugonic human type. These variants are designated 'dysgonic human'.

2. A few bovine strains from human beings and pigs have grown rather better on glycerin-egg and agar than any strain obtained from bovine tuberculous lesions, though not so well as eugonic human strains. The retention of the eugonic character of the strains after repeated passage of the bacilli through rabbits showed that the luxuriance of growth was not due to an admixture of human bacilli. These strains are designated 'eugonic bovine'.

3. A few strains have been described which, although they were typically avian in respect of virulence, produced in culture dry coherent growths resembling those of mammalian bacilli. Two of eleven strains from fowls investigated by Weber and Bofinger (1904), and two obtained from captive birds by M. Koch and L. Rabinowitsch (1907) grew in this way. A. S. Griffith (1925[2]) isolated from a sheep with progressive general tuberculosis a virulent avian strain which produced dry crumbly layers on glycerin-agar and potato. A. Eastwood and F. Griffith (1914) obtained from a pig a strain which in culture was dry and eugonic, resembling the human type of bacillus, but which was not pathogenic for the guinea-pig and produced avian tuberculosis in the rabbit. This strain was of low virulence for the fowl.

BIOCHEMICAL REACTIONS.

The glycerin broth reaction curve. In 1903 a biochemical test for distinguishing human from bovine tubercle bacilli was introduced by Theobald Smith, who stated that when tubercle bacilli are grown on the surface of glycerin broth with an initial acidity, with phenol-phthalein as indicator, the subsequent changes in the reaction of the broth differ with the type of bacillus. He found that with cultures of bovine tubercle bacilli the acidity of the medium steadily diminished until the neutral point was reached or even passed. With cultures of human tubercle bacilli there was first a diminution in acidity, not, however, so great as with bovine bacilli, and then a reversal of the reaction, although the final compared with the initial reaction always showed a diminished acidity.

A. S. Griffith (1907) and others repeated Theobald Smith's experiments and confirmed the observation that there are two contrasted reaction curves, one in which the end reaction was near the neutral point, the other in which, after a preliminary advance in the alkaline direction, there was a development of acidity, the final reaction often being more acid than the initial. But all strains of tubercle bacilli did not react in one or other of these two ways. Between the two extremes there was every grade of intermediate reaction and nowhere a gap to indicate that two biochemically different classes of tubercle bacilli were being dealt with. A. Harden (1913) was unable to find any physiological difference between tubercle bacilli of different origins. The test is difficult and laborious and requires a long time for its performance. For the above reasons the glycerin broth test cannot be substituted for the simpler and more certain cultural and pathogenic tests in the differentiation of human from bovine tubercle bacilli. When glucose was substituted for glycerin all strains reacted in the same way, the broth becoming strongly alkaline.

A. S. Griffith (1907) found that glycerin litmus milk could be used instead of glycerin broth. Strains which produced thick pellicles caused the milk first to turn red and then to clot. When the pellicle was scanty the milk became alkaline.

O. Bang (1907) investigated the Theobald Smith reaction curve of the avian bacillus. He found that the glycerin broth upon which the avian bacillus is forming a pellicle steadily diminishes in acidity and finally becomes strongly alkaline.

SEROLOGICAL REACTIONS.

It has not been found possible to differentiate between human and bovine bacilli by agglutination reactions. According to the results of Tulloch *et al.* (1924), G. S. Wilson (1925) and A. S. Griffith (1925^1), bovine and human strains are serologically identical, since all bovine and human strains were able to absorb the agglutinin both from human and from bovine sera. Typical avian strains produce a specific agglutinin with which human and bovine strains do not combine. It is possible, therefore, to distinguish the common avian bacillus from mammalian bacilli by the absorption of agglutinin test.

REFERENCES.

BANG, O., 1907, *Zbl. Bakt.*, Abt. I, Orig., **43**, 34.
DORSET, M., 1902, *Amer. Med.*, **3**, 555.
EASTWOOD, A. & GRIFFITH, F., 1914, *Rep. Loc. Govt. Bd. Publ. Hlth.*, n.s., No. **91**.
FROUIN, A. & GUILLAUMIE, M., 1923, *C.R. Soc. Biol.*, Paris, **90**, 831.
GRIFFITH, A. S., 1907, *Royal Commission on Tuberculosis*, 2nd Interim Rep. Appendix, **3**, 53 and 85 ; 1911, *ibid.*, Final Rep. Appendix, **1**, 26 ; 1916, *Lancet*, Lond., i, 723 ; 1925^1, *Tubercle*, Lond., **6**, 417 ; 1925^2, *J. Comp. Path.*, **38**, 157.
GRIFFITH, A. S. & F., 1907, *Royal Commission on Tuberculosis*, 2nd Interim Rep. Appendix, **3**, 1.
GRIFFITH, F., 1911, *Royal Commission on Tuberculosis*, Final Rep. Appendix, **4**, 178.
HARDEN, A., 1913, *Royal Commission on Tuberculosis*, Final Rep. Appendix, **6**, 1.
KOCH, M. & RABINOWITSCH, L., 1907, *Virchows Arch.*, **190**, 246.
KOCH, R., 1884, *Mitt. GesundhAmt.*, **2**, 49.
NOCARD & ROUX, 1887, *Ann. Inst. Pasteur*, **1**, 19.
NOVY, F. G. & SOULE, M. H., 1925, *J. Infect Dis.*, **36**, 168.
SMITH, TH., 1905, *J. Med. Res.*, **13**, 253 and 405.
SMITH, TH. & BROWN, H. R., 1907, *J. Med. Res.*, **16**, 435.
TULLOCH, W. J., *et al.*, 1924, *Tubercle*, Lond., **6**, 18, 57 and 105.
UHLENHUTH, 1909, *Zbl. Bakt.*, Abt. I, Orig., **42**, Beiheft, 62.
WEBER, A. & BOFINGER, 1904, *Tuberk. Arb.*, Berl., **1**, 83.
WILSON, G. S., 1925, *J. Path. Bact.*, **28**, 69.

Experimental Tuberculosis.

By A. Stanley Griffith.

METHODS OF EXPERIMENTAL INFECTION.

Tuberculosis can be produced experimentally in susceptible animals by inoculation into any tissue or by any natural route of infection. For virulence tests the intravenous (intraperitoneal in the guinea-pig) and subcutaneous methods are used by the majority of workers. There is no advantage, for example, in using the intra-articular or the intra-ocular methods, which cause unnecessary pain and distress to the animal. For comparing the susceptibility of different species to a given strain of bacilli one of the natural routes of infection—feeding, inhalation or eye instillation—should be chosen.

SUSCEPTIBILITY OF THE VARIOUS EXPERIMENTAL ANIMALS TO MAMMALIAN AND AVIAN TUBERCLE BACILLI.

No animal is completely immune against experimental tuberculosis, but the different species vary widely in their degree of susceptibility to the different types of tubercle bacilli. Some species of animal, e.g. pigs, parrots and rabbits, are susceptible, though not in equal degree, to all three types of bacillus (human, bovine and avian). Guinea-pigs and monkeys are highly susceptible to mammalian bacilli but are completely resistant to avian bacilli. Fowls are readily infected with avian bacilli but are quite resistant to mammalian bacilli. Two species of animals—rats and mice—are highly resistant to all three types and develop progressive tuberculosis only when large doses of the bacilli are introduced into the blood-stream or the peritoneal cavity. Bovine animals, goats, sheep, horses, pigs and cats are highly susceptible to bovine bacilli and very resistant to human bacilli.

Mammalian tubercle bacilli. According to their susceptibility to the two mammalian types of bacillus the different species of animals can be divided into three groups. In each of two of the groups the animals react in much the same way to either type of bacillus, one group containing the animals which are highly susceptible, the other the animals which are very resistant. The animals in the third group are susceptible to one type of bacillus but resistant to the other.

The susceptible group includes apes, monkeys and guinea-pigs. Either type of bacillus invariably produces in these species of animals fatal general progressive tuberculosis howsoever inoculated and in whatsoever dose. For the guinea-pig, the bovine bacillus is, as a rule, slightly more virulent than the human bacillus, generally producing a more acute tuberculous process with more rapid and complete caseo-necrosis of the lesions; these differences, however, are not constant.

The resistant group contains the dog, the rat and the mouse. The dog and the rat are highly resistant to the subcutaneous inoculation of large doses and to the intraperitoneal inoculation of small doses of culture.

When large doses are inoculated intraperitoneally (or intravenously), both types of bacillus multiply freely in the tissues of these animals and cause their death from toxæmia without producing much tissue destruction or lesions typical of tuberculosis. Tissue changes are more marked in the dog than in the rat. The mouse closely resembles the rat in its reactions but offers less resistance to the growth of the bacillus in its tissues. Trommsdorff (1909) concluded from his experiments that the two types of bacilli could be differentiated by intravenous inoculation of mice, but Lange (1922) was unable to confirm this.

None of the species, either in the resistant group or in the susceptible group, can be used for differentiating between the two types. For this purpose one or other of the species in the third or differential group must be employed. The species of animals which are susceptible to the bovine bacillus and resistant to the human bacillus are the calf, rabbit, pig, goat, cat, sheep, horse and ferret. Of these the rabbit is the most convenient and is now generally employed. The work of the German and English Commissions showed that there is complete parallelism between the results produced by the two types in the calf and rabbit. When using the rabbit for the purpose of determining the type of a strain it must be remembered that this animal is not insusceptible to the human bacillus ; severe and acute or slowly progressive tuberculosis may be produced by intravenous and even by subcutaneous inoculation. It has also to be remembered that bovine bacilli do not always produce rapidly fatal tuberculosis in the rabbit when small doses are inoculated subcutaneously. Thus, in order to bring out most clearly the differences in virulence for the rabbit of the two types, the doses used for the different methods of inoculation must be carefully estimated. If the intravenous method is used the dose of culture should not exceed 0·01 mgm., because larger doses of human bacilli may give rise to an acute tuberculosis which is difficult to distinguish from that set up by bovine bacilli. For subcutaneous inoculation the best dose is 10 mgm. Rabbits inoculated intravenously with 0·01 mgm. or subcutaneously with 10 mgm. of bovine tubercle bacilli invariably die of progressive general tuberculosis within about 5 weeks after intravenous and 10 weeks after subcutaneous inoculation. There has been no death from tuberculosis after the intravenous inoculation of 0·01 mgm., or the subcutaneous inoculation of 10 mgm. of human tubercle bacilli within the period of observation of 3 to 4 months.

The calf is more resistant to both types of bacilli than the rabbit. The virulence of a strain is best tested by the inoculation of 50 mgm. of culture subcutaneously. This was the method recommended by the German Commission and was adopted by the English Commission. A calf inoculated with 50 mgm. of young serum-grown bovine bacilli invariably develops general tuberculosis, which, except in highly resistant calves, is progressive and causes death within about 10 weeks. The human tubercle bacillus, on the other hand, in the same or much larger dose is incapable, when inoculated subcutaneously, of setting up progressive tuberculosis.

The goat is an excellent animal for differentiating between bovine and human strains. Comparative experiments have shown that to the bovine bacillus the young goat is more susceptible than the calf and to the human bacillus more resistant than the rabbit. Adult goats are more resistant than kids. Progressive tuberculosis has never been produced in the goat by human bacilli. Calmette and Guérin (1909) consider that the lactating goat is an excellent means of differentiation. If bacilli of human origin are injected into the mammary cisterns the infection, though persistent, remains local. If, on the other hand, bacilli of bovine type are injected, the tuberculous infection, at first circumscribed, quickly spreads to other parts of the body and causes the death of the animal in 4 to 5 months from generalized tuberculosis.

The pig, the cat, or any of the other species in this group can equally well be used for differentiating bovine from human strains, but for various reasons are less suitable. In these species of animals the bovine bacillus readily produces general progressive tuberculosis, while the human bacillus is incapable of producing more than slight retrogressive disease.

Avian bacilli. All species of birds can be infected experimentally with avian tubercle bacilli. The common fowl is highly susceptible and develops generalized progressive tuberculosis after inoculation or feeding with extremely small doses of bacilli. Pigeons are much more resistant than fowls both to inoculation and feeding, and are unsuitable for testing virulence. Parrots are highly susceptible to experimental infection with avian bacilli.

Of the common laboratory mammals the most susceptible to avian bacilli are the rabbit and the mouse. In the rabbit the effects produced are so characteristic that this species may be used in place of the fowl to confirm a diagnosis of avian infection based on cultural characters. Experimentally, the avian bacillus is pathogenic for many other species of mammals, but does not produce in them progressive tuberculosis. After intravenous inoculation of moderate doses, some species of animals (the calf, pig, horse, monkey, cat and guinea-pig) succumb almost invariably to the toxic effects of the bacilli multiplying in the tissues. After subcutaneous inoculation or feeding the bacilli give rise to necrotic lesions at the points of entry and in the nearest glands. These lesions subsequently soften and ulcerate or undergo calcification. After both these methods of infection, avian bacilli are disseminated over the body and multiply for a time in the organs and glands, where they may produce small lesions and are recoverable after periods ranging up to six months. Ultimately the bacilli are eliminated or they die and the animals make a complete recovery. At the autopsies disseminated lesions are always few in number and take the form of purulent or calcareous foci. Fatal results after subcutaneous inoculation or feeding are exceptional and have been recorded only in the young of the goat (F. Griffith, 1911; O. Bang, 1913), and in the foal (O. Bang, 1913).

The dog has been shown to be refractory to avian bacilli inoculated intravenously, intraperitoneally or subcutaneously. A chimpanzee completely resisted the subcutaneous inoculation of 50 mgm. of avian bacilli. The rat reacts to avian in much the same way as to mammalian bacilli.

DIFFERENCES IN PATHOGENICITY BETWEEN AVIAN AND MAMMALIAN TUBERCLE BACILLI.

The avian tubercle bacillus in extremely small dose can produce in all species of birds progressive general tuberculosis, whether inoculated into the tissues or given with the food. Mammalian tubercle bacilli are not pathogenic for birds—with the exception of parrots—and are incapable of producing progressive tuberculosis in them, no matter how large the dose. The avian bacillus inoculated subcutaneously in very large dose does not produce progressive tuberculosis in guinea-pigs, whereas mammalian bacilli in extremely small dose always give rise to progressive general tuberculosis.

The avian bacillus can produce progressive tuberculosis in rabbits by inoculation and by feeding, and in virulence for this species is intermediate between bovine and human bacilli. It is possible to distinguish the avian type from the bovine and human types by the effects they severally produce in the rabbit, but it is necessary for the purpose to inoculate more than one rabbit with estimated doses by different methods. After intravenous inoculation of moderate doses (0·1 to 1·0 mgm.) of avian bacilli an acute tuberculosis results, which has definite points of difference from that which follows inoculation of bovine bacilli. The most striking of these is in the condition of the lungs, which after avian bacilli are crepitant, œdematous and contain few tubercles, and after bovine bacilli are consolidated and packed with coalescing caseating tubercles. Avian bacilli in small dose intravenously, or in moderate dose subcutaneously or by feeding, produce in rabbits chronic tuberculosis with characteristic distribution ; the seats of election of the lesions are in the joints, testes and areolar tissues ; the lungs, kidneys and glands are rarely severely affected and the liver and spleen are often free from lesions. Bovine bacilli, on the other hand, cause severe and characteristic tuberculosis of the lungs and kidneys, and the glands frequently contain caseous lesions. Sometimes bovine bacilli cause a chronic form of tuberculosis which has a great resemblance to that produced by avian bacilli. There is the same tendency for joints, testes and areolar tissues to become the seats of tuberculosis, but never to so marked an extent as after avian bacilli. Avian bacilli, however, never give rise to tuberculosis of the eyes, caseopus in the pelvis of the kidneys or massive caseous pneumonia, conditions which are so frequently seen in the chronic bovine tuberculosis of rabbits.

Human tubercle bacilli may, after intravenous inoculation, produce in rabbits an acute tuberculosis resembling that which follows inoculation of bovine bacilli, though multiplication of bacilli is not so marked a feature, but more usually a chronic form of disease results with lesions in the joints and areolar tissues ; the almost invariable occurrence also of lesions in the eyes and muscles and of caseo-pus in the pelvis of the kidneys distinguishes human from avian infections. After subcutaneous inoculation of human bacilli the tuberculous disease is generally restricted to the seat of inoculation ; when there are disseminated lesions they are found most often in the lungs and only occasionally in the kidneys ; the joints, in the writer's experience, are never affected.

Avian bacilli multiply more abundantly in the tissues of the rabbit than bovine bacilli.

Other mammals are not useful for differentiating avian from mammalian bacilli. The avian bacillus is pathogenic for the mouse, but its effects are essentially the same as those of mammalian bacilli.

For the diagnosis of a strain of tubercle bacilli by animal tests alone it would be sufficient to employ the guinea-pig, the fowl and the rabbit.

DISTRIBUTION OF LESIONS.

The distribution of the lesions in experimental tuberculosis varies with the mode of injection, the species of animal, the type of bacillus and the duration of life.

Intravenous inoculation of virulent bacilli into susceptible animals always produces a very severe type of tuberculosis. The bacilli are distributed by the blood to the various organs and glands, where they settle and multiply. If the dose is a large one the animal may die from the toxic effects of the bacilli multiplying in the tissues, without the formation of lesions. This acute septicæmic form of tuberculosis—the 'tuberculose septicémique' of French authors, first described by Yersin (1888) and known as the Yersin type of disease—is met with most commonly in animals, especially rabbits, inoculated intravenously with large doses of avian bacilli. Acute septicæmic tuberculosis without visible lesions is not often produced in animals inoculated with ordinary doses of mammalian tubercle bacilli. (Exceptions are kittens, rats and mice.)

When susceptible animals are inoculated intravenously with moderate doses of mammalian bacilli, a rapidly fatal generalized miliary tuberculosis is produced, which is always of great and generally of greatest severity in the lungs. Rabbits, for example, inoculated intravenously with 0·01 mgm. of bovine bacilli die within about 30 days. The lungs are enlarged, almost completely consolidated and closely beset with small caseating tubercles ; the liver and the swollen spleen are peppered with fine grey tubercles ; the kidneys, marrow and lymphatic glands contain a moderate number of tubercles.

Intravenous inoculation of bacilli of relatively low virulence may, if the dose is large, cause death from pneumonic consolidation due to the irritant and toxic action of the bacilli, as, for example, in calves inoculated with human bacilli. In rabbits the intravenous inoculation of human bacilli in doses of 1·0 or 0·1 mgm. may cause acute general miliary tuberculosis, but usually the chronic forms of tuberculosis are produced, with localizations in the bones or joints, eyes, testes, muscles, mammary glands or skin. After 0·01 mgm. of human bacilli intravenously a rabbit has not in the writer's experience died of tuberculosis within 3 months, though death has occurred at a later period from chronic general tuberculosis; the majority overcome the infection and when killed 3 to 4 months later show slight tuberculosis limited to the lungs or the lungs and kidneys.

There is a striking difference in the distribution of the lesions in mammals after intravenous inoculation of mammalian bacilli and in fowls after intravenous inoculation of avian bacilli. In mammals the lungs as well as other organs and glands are beset with miliary tubercles. In the fowl the lungs contain perhaps a tubercle or two and the kidneys are free from lesions, while the liver and spleen are greatly enlarged and thickly beset with miliary tubercles.

In the rabbit the effects produced by avian bacilli are different from those of mammalian bacilli. The disease-picture varies with the duration of life and generally corresponds to one or other of three forms. The first form is the ' Yersin type ' and is found in rabbits which die in 2 to 4 weeks. At the autopsy the lungs are œdematous, the liver and spleen are enlarged and there is general anæmia ; no tubercles are visible to the naked eye in the organs, but smear preparations show extremely numerous bacilli in spleen, liver and marrow.

The second form of disease presents the picture of acute general miliary tuberculosis and is seen in animals which die of tuberculosis in from 4 to 12 weeks. The spleen is greatly, sometimes enormously, enlarged, and is beset with tubercles, confluent in places ; the liver shows numerous tubercles ; the lungs are œdematous and usually show a few caseating tubercles or small marginal patches. The thoracic, portal and other glands, the areolar and retroperitoneal tissues, the appendix and sacculus rotundus, omentum and mesentery also show miliary caseous tubercles. The third form of disease runs a chronic course and is described under ' subcutaneous inoculation '.

Intraperitoneal inoculation produces the same forms of general tuberculosis as intravenous, but the process is generally less acute. The bacilli are in part rapidly absorbed into the general circulation and in part retained in the omentum or on the peritoneum. The omentum contracts and becomes a mass of tuberculous caseating tissue which adheres to surrounding structures, and the peritoneum becomes covered with tubercles.

Subcutaneous inoculation. When tubercle bacilli are inoculated subcutaneously they remain for the most part lodged in the subcutaneous tissues, where they give rise to a caseo-necrotic tumour which varies in rapidity of development, in size and appearance according to the number and relative virulence of the bacilli and the species of animal. Very soon after inoculation bacilli begin to invade the adjacent lymphatic glands, which progressively enlarge and become wholly or in part converted into caseous or caseo-necrotic substance. Both at the seat of inoculation and in the adjacent glands the tuberculous process may extend by contiguity into the adjacent structures—muscles, skin and lymphatic vessels.

When moderate or large doses of bacilli are injected a certain number enter the circulation within the first few days and are distributed over the body. It was ascertained experimentally by A. S. and F. Griffith (1907) that this initial dispersal occurred in all species of animals whether the bacilli were virulent or not for those species. In animals highly susceptible to the bacilli inoculated, the amount of this dissemination by the blood largely determines the severity and acuteness of the resulting disease, which in calves and rabbits may be similar to that following intravenous inoculation of bacilli, all the organs and glands showing miliary tubercles.

If the number of bacilli disseminated by the blood in the early stages of the infection is too small to cause a rapidly fatal miliary tuberculosis and the animal survives to die after several weeks of progressive tuberculosis, one sees in the organs, in addition to miliary tubercles, tuberculous nodules and masses of varying size, which have a characteristic appearance and distribution in each species of animal. In the guinea-pig the brunt of the disease is always borne by the liver and spleen, which generally show the well-known peculiar and characteristic ramifying or diffuse necrotic or caseo-necrotic tracts. The lungs contain a variable number of miliary tubercles and in the more chronic cases may be extensively replaced by caseating tuberculous masses. The kidneys always suffer the least severely and are often quite unaffected. The lymphatic glands of the guinea-pig are very susceptible and generally all are enlarged, showing varying degrees of caseation. After subcutaneous inoculation of avian culture in doses up to 100 mgm. the guinea-pig, according to the majority of observers, does not develop general progressive tuberculosis. Purulent foci are seen at the seat of inoculation and in the adjacent glands, but no lesions similar to those of mammalian tuberculosis ever develop in the organs. In the monkey, tuberculosis produced by mammalian bacilli has a very similar distribution to that in the guinea-pig, but the lesions in the liver and spleen take the form of caseo-purulent abscesses and miliary caseous tubercles instead of diffuse infiltrating areas.

In the calf, pig, goat, sheep, horse, cat and rabbit, bovine bacilli in adequate dose produce rapidly fatal general progressive tuberculosis. In these species the lungs are always severely implicated; the kidneys suffer much less. In the calf, at the seat of inoculation a dense necrotic tumour develops which infiltrates skin and subjacent muscles. The nearest glands are much enlarged and uniformly dense and necrotic. The thoracic, portal, mesenteric and cœliac are also much enlarged, the cortices are uniformly tuberculous but less advanced in caseo-necrosis than the regional glands; all other lymph and hæmolymph glands are beset with caseous tubercles. Of the organs the lungs are affected with the greatest severity and are large, dark red, almost completely airless and packed with miliary caseating, coalescing tubercles. The spleen and liver are also closely beset. The kidneys contain less numerous tubercles.

In rabbits which die of tuberculosis 6 to 8 weeks after inoculation, the lungs are greatly enlarged, almost entirely airless and composed in great part of firm reddish-grey tissue infiltrated with irregular caseous areas of various sizes; the rest of the lung contains caseating tubercles mainly aggregated and coalescing, surrounded by œdematous, emphysematous and congested lung tissue. The kidneys show in the cortices numerous caseating nodules 1 to 3 mm. in diameter, the larger projecting from the surface and extending deeply into the substance. The liver and spleen contain small scattered tubercles. Generally all the lymph glands contain caseous tubercles, and tubercles are sometimes found in the heart, thyroid, suprarenals, intestines and omentum. In the more chronic forms of tuberculosis found in rabbits which live three months or longer, the tuberculosis internally is often confined to the lungs, kidneys and lymphoid tissue of the appendix and sacculus rotundus. The lungs are in a condition of massive caseating pneumonia and the kidneys are beset with nodules projecting considerably from the surface. In these chronic forms of tuberculosis the joints and eyes of the rabbit are frequently affected.

In the goat the lesions in the lungs are very prone to break down with the formation of cavities. In kittens, bovine bacilli multiply so abundantly in the tissues as sometimes to cause death before lesions become visible to the naked eye.

To the human tubercle bacillus the calf, goat, pig, cat, horse and sheep are highly resistant and never develop more than local or slight general retrogressive tuberculosis. In heifer calves the human bacilli may be excreted into the milk sinuses of the undeveloped mamma where they may live and multiply until such time as the heifer gives birth to a calf. Cows inoculated subcutaneously (or intravenously) with human bacilli may excrete the bacilli in the milk for a long time subsequently.

The rabbit, as already mentioned, is less resistant to human bacilli than the calf, and after subcutaneous inoculation of 10 mgm. or more may develop slowly progressive tuberculosis. Usually, however, the tuberculosis is either limited to the seat of inoculation or there is slight extension to the lungs, and, occasionally, the kidneys; the nearest glands are affected, usually slightly, in about 40 per cent. of the cases. Pulmonary tuberculosis in the rabbit may be slight, moderate or severe, but in the greater proportion of instances it is slight and in the form of tubercles and irregular grey, centrally caseous nodules, mainly subpleural or marginal. If rabbits with moderate or severe tuberculosis are allowed to live, the pulmonary lesions may become converted into cysts or cavities.

The chronic tuberculosis produced in rabbits by the subcutaneous inoculation of avian bacilli has many points of resemblance to chronic mammalian tuberculosis. The most characteristic feature of chronic avian tuberculosis in the rabbit is the affection of the bones and joints. If the rabbit lives longer than three months many of the limb joints are certain to be found tuberculous. The process appears to begin in the synovial membrane, but the heads of the long bones may be affected, showing caseo-necrosis. Joint disease in the rabbit produced by human bacilli begins in the synovial membrane, whereas that produced by bovine bacilli is usually preceded by tuberculosis of the ends of the bones.

There are striking differences in the distribution of the generalized lesions in chronic (bovine) tuberculosis in the rabbit and in the guinea-pig. In the rabbit, the spleen, liver and lymphatic glands are unaffected or show insignificant lesions, while the lungs and kidneys are extensively tuberculous. In the guinea-pig, on the other hand, the liver and spleen bear the brunt of the disease. Another striking distinction between tuberculosis in the guinea-pig and the rabbit is that in the former lesions rarely, if ever, occur in the bones, joints, eyes and mammary glands or on the pleura, whereas in the rabbit these structures are extremely liable to tuberculosis. The pleura of the rabbit often becomes covered with fibro-caseous sequin-like tubercles or plaques, and masses of fibro-caseous substance may develop along the ribs and on the pericardium.

Inhalation. The experiments of many observers have shown conclusively that pulmonary tuberculosis is readily produced in susceptible animals by making them breathe air containing tubercle bacilli in suspension, either as dust or in spray from a Buchner apparatus. When the infective particles are of great fineness they are drawn deeply into the lungs and may be demonstrated in the thin marginal portions a few minutes after exposure to the spray (Cobbett, 1910). The proportion of the bacilli inhaled which reach the alveoli and bronchioles is directly related to the fineness of the dust or spray. Lange and Nowoselsky (1925) estimate that with moist spray an average of 21·5 per cent., with dry dust 12 per cent., reach the lung alveoli. When the particles are gross they are for the most part arrested on the mucous membrane of the nose and pharynx, and in these circumstances the bacilli may find their way into the neck glands and produce a primary cervical adenitis.

With regard to the number of bacilli which suffice to infect by the respiratory route, there is every reason to believe from the careful experiments of B. Lange (1926) that one virulent bacillus is sufficient.

After infection by inhalation, pulmonary lesions in the guinea-pig become visible to the naked eye on about the ninth day (Cobbett). The bronchial glands, though they constantly contain tubercle bacilli from the fifth day onwards, do not become definitely enlarged until the twelfth day, or show lesions until the twenty-third day. The tuberculosis produced in guinea-pigs and monkeys by inhalation rapidly becomes generalized and is invariably fatal; in monkeys the process extends locally and quickly implicates the whole of the lobe. The intestines and mesenteric glands escape infection altogether in the early stage of the disease and are only affected later as part of a generalized tuberculosis.

The condition of the lungs and bronchial glands varies with the number of bacilli inhaled. If the dose is massive a caseous pneumonia is the result. With moderate doses many discrete uniformly distributed primary foci may develop. In such circumstances the bronchial glands on both sides become enlarged. With extremely small doses, as is frequently the case in natural infections, only a single lesion, which may be found in any part of the lung, may arise. When there is a single pulmonary lesion the bronchial gland draining that region is invariably larger and more advanced in caseation than any of the other bronchial glands. The association of a primary focus in the lung and enlarged bronchial gland on that side is known as the primary pulmonary complex (Parrot, 1876 ; Küss, 1898) or ' lesions of primary localization ' (Cummins, 1924).

Ocular instillation. Instillation of tubercle bacilli into the conjunctival sac, a method first employed by Calmette, Guérin and Grysez (1913), represents a natural form of infection and is very useful for testing the resistance of animals such as guinea-pigs and monkeys to tuberculosis after any form of treatment. If a minute drop of fluid containing tubercle bacilli is dropped upon the eyeball of a guinea-pig or a monkey, a typical cervical adenitis is produced, which after moderate doses is almost always accompanied by inflammatory specific changes about the eye or in the nose. The eye changes are ulceration of the conjunctiva, thickening of the eyelids, infiltration of the tissue about the globe and corneal opacity. The nasal mucous membrane and bones are sometimes involved, the nasal cavity becoming filled with caseo-pus. With small doses or with attenuated bacilli local changes are less frequent and intense, and the glandular enlargement is more indolent.

The first gland to become palpable is generally the submaxillary gland ; in the monkey the parotid gland is often the earliest to enlarge. The retropharyngeal and cervical glands quickly follow and together they form a mass in the neck which causes obstruction to respiration and deglutition. Sometimes the intestines and mesenteric glands show lesions ; these are caused by bacilli which have been carried down with the lachrymal secretion and swallowed. In guinea-pigs, after moderately large doses, the lungs sometimes show caseous nodules or pneumonic patches and the tracheo-bronchial glands are considerably enlarged. These changes are probably due to direct infection, i.e. by insufflation, and not to lymphatic spread from the neck glands. This complication has not been observed in monkeys.

Feeding. The introduction of tubercle bacilli into the alimentary tract is not always followed even in the most susceptible animals by the development of tuberculosis. The injected bacilli may either pass straight through the canal or, if they penetrate the mucous membrane, be taken up by phagocytes and destroyed.

When tubercle bacilli are absorbed from the alimentary tract and escape immediate destruction, they either remain in the mucous membrane and set up characteristic tuberculous lesions, or they pass through the mucous membrane into the nearest glands, leaving no trace of their passage. In experiments carried out for the Tuberculosis Commission (A. S. Griffith, 1907), tubercle bacilli were found in the mesenteric glands of some dogs 3½ to 5 hours after their introduction into the alimentary canal. In the pig they were found in the glands within 24 hours. Possibly there are differences in permeability of the membranes, as well as in rapidity of transit from the canal to the glands in different species of animals, but exact observations have not been made on these points. Having reached the glands the great majority of the bacilli are arrested, but some, after a period which appears to vary with the species of animal used, are carried onwards by the lymph and blood-streams into the lungs and even beyond. In two instances (a dog and a monkey) tubercle bacilli were found to be present in the lungs within four days of ingestion, and in other instances within a week, that is, after periods too short for an active dissemination from a tuberculous focus to have taken place. These experiments showed that the lymphatic glands, like the mucous membrane of the alimentary tract, are not an absolute barrier to the direct passage of ingested bacilli from the alimentary canal to the lungs and other organs.

The local effects produced by ingested tubercle bacilli differ according to the species of animal and the virulence of the organism.

In chimpanzees and the various species of monkeys, which are very susceptible to infection with both types of mammalian tubercle bacilli, the mucous membrane of the alimentary tract is specially liable to attack, and tuberculous ulcers may be formed along the whole length of the small and large intestines. The tonsils also are frequently attacked and may be destroyed by caseo-necrosis. The regional lymphatic glands always become enlarged and caseous and frequently suppurate ; sometimes by fusing together they form very large tumours both in the neck and in the mesentery. In baboons a striking feature is the frequency with which the lymphatic vessels in the mesentery, extending between an ulcer and the enlarged glands, become tuberculous. This tuberculous lymphangitis may be succeeded by great thickening and extensive tuberculous infiltration of the mesentery itself, with secondary ulceration of the mucous membrane of the bowel attached to it. In the baboon, caseous nodules and typical tuberculous ulcers have been found in the stomach. In guinea-pigs and rabbits (bovine infections) lesions are generally found in the intestines when doses of moderate size are given ; they are situated in the lymphoid patches and follicles, both of the small and large intestines ; the pharyngeal mucous membrane is not frequently affected.

In pigs the tonsils and pharyngeal mucous membrane are more frequently affected than the intestines. The intestinal mucous membrane does not often show lesions and when present they are generally inconspicuous.

In the calf the small intestines are very susceptible to bovine tubercle bacilli, and ulcers and submucous nodules may be found along the whole length of the gut ; nodules have also been found in the wall of the stomach. The pharyngeal mucous membrane and the tonsils are not frequently involved. In the goat fed with bovine bacilli, typical tuberculous lesions have been seen in the tonsils, stomach and small intestines. In cats and dogs the alimentary mucous membrane rarely shows lesions. In the pig, calf and goat, human tubercle bacilli do not produce lesions in the mucous membrane, but only retrogressive calcareous lesions in the adjacent glands. In fowls and rabbits, avian tubercle bacilli produce in the intestines characteristic nodular outgrowths which are often merely saccular dilatations of the wall, lined internally with caseous substance (see page 201).

In all species of mammals highly susceptible to the bacilli introduced, infection from the alimentary tract is invariably followed by enlargement and caseation of the neck or mesenteric lymphatic glands and generalization of the disease. In the more resistant animals (calf, goat, pig, cat, dog) the tuberculosis may be limited to the glands of the alimentary tract.

The course of the disease in progressive cases is more protracted than after subcutaneous inoculation, but the distribution of the disseminated lesions is the same. The bronchial glands share in the general tuberculosis and are always in a less advanced stage than the mesenteric or neck glands.

Summary. The most important point brought out by the study of the distribution of lesions in experimental animals is that for each form of infection (except intravascular) there is a definite and distinct localization of the primary lesions, whether the dose is large or small. In experimental tuberculosis, therefore, the law of Cohnheim holds good. At whatever point the tuberculous virus is introduced or penetrates an epithelial surface, there, or in the adjacent lymphatic glands, a tuberculous lesion appears. The primary localization of the lesions is determined by the portal of entry of the bacilli. The fact that in susceptible animals the primary localization of the lesions always indicates the avenue of infection is of the greatest value in experimental work on tuberculosis, since one can judge whether or not the tuberculous lesions found at autopsy in an experimental animal were produced by the material inoculated. If, for example, after subcutaneous inoculation a tracheo-bronchial gland tuberculosis is found with no disease of the glands in the groin, one may conclude that the infection was spontaneously acquired.

The law of Cohnheim is not invalidated by absence of lesion at the point of penetration of the bacilli. In conjunctival instillations and in feeding experiments it frequently happens that bacilli pass through the mucous membrane without leaving trace of their passage, but the glands in the neck or in the mesentery always show lesions at a more advanced stage than any other regional glands. There is also evidence that when large doses of virus are fed to animals, tubercle bacilli may occasionally and in small numbers pass through the lymphatic glands, or second line of defence, directly into the general circulation. But the greater number are arrested in the neck or mesenteric glands, where, if the animal is permitted to live, they give rise to obvious lesions which mark the portal by which they entered.

EFFECTS OF VARIATION IN INFECTIVE DOSE AND OF CHANNEL OF ENTRY.

The effects produced in an animal depend upon several factors of which the most important are: (1) the virulence of the strain, (2) the number of living bacilli introduced, (3) the mode of infection, and (4) the susceptibility of the animal.

In animals which are highly susceptible to the bacilli introduced, for example, monkeys and guinea-pigs to mammalian tubercle bacilli, rabbits to bovine bacilli, fowls to avian bacilli, the end result is the same whatever the dose (one virulent bacillus is sufficient) or the channel of infection. In these species of animals invasion is always followed by generalization and death from progressive tuberculosis, which is acute or more chronic according to the number of bacilli inoculated.

The number of living bacilli inoculated has most importance in animals which possess some moderate power of resistance to tuberculosis. In the calf, which may be taken as representing this resistant class of animals, small doses of virulent bovine bacilli inoculated subcutaneously or given with the food, often produce only limited and localized lesions, which become retrogressive. Medium-sized doses (10 mgm. subcutaneously) give irregular results, some calves dying of fatal general tuberculosis, others surviving and showing at autopsy after 3 to 4 months different degrees of general progressive and retrogressive tuberculosis. Large doses (50 mgm. subcutaneously), on the other hand, invariably give rise to general tuberculosis, which, except in a small minority of cases, is progressive and fatal within three months. Much larger doses are necessary to produce rapidly fatal general progressive tuberculosis in calves by feeding than by subcutaneous inoculation. On the other hand, extremely small doses suffice to cause rapidly fatal general progressive tuberculosis when inoculated intravenously.

In highly resistant animals, for example, the rat to all three types, or the calf to the human type, or the dog to mammalian bacilli, large doses subcutaneously are not more effective than small doses. To overcome the resistance of these species it is necessary to inoculate large doses of the bacilli intravenously or intraperitoneally.

TABLE I.

The chief Characters and Properties of the Bovine, Human and Avian Types of Tubercle Bacillus.

	Cultural Characteristics.		Pathogenicity.	
	Bovine Serum.	Glycerin Media.		
Bovine type.	Thin grey, or greyish white and not pigmented.	The great majority of the strains grow with difficulty and slowly, i.e. are dysgonic ; a few grow with relative luxuriance, though not equal to human (eugonic) strains.	Virulent for all species of mammals, including man.	The dog can be infected, but shows a high degree of resistance to both types. All species of birds, except the parrot, are refractory.

TABLE I—*continued.*

	Cultural Characteristics.		Pathogenicity.	
	Bovine Serum.	Glycerin Media.		
Human type.	Growth more abundant than with bovine strains, often showing yellow pigment.	All strains grow readily, producing creamy wrinkled or warty layers, i.e. are eugonic.	Virulent for the ape, monkey and guinea-pig, producing general progressive tuberculosis; slightly virulent for the rabbit, disease produced usually limited and retrogressive, but sometimes slowly progressive. Not virulent for the calf, goat, pig, horse and sheep.	The dog can be infected, but shows a high degree of resistance to both types. All species of birds, except the parrot, are refractory.
	Human and bovine cultures are dry, coherent and not readily emulsified.			
Avian type.	Thin, moist and transparent.	More abundant than on media without glycerin. Old cultures have a yellow or reddish yellow colour.	Virulent for all species of birds. Of mammalian species the rabbit is susceptible both to experimental and natural infection. The sheep, pig, possibly the horse, and some wild mammals in captivity are susceptible to natural infection. The dog, the ape and the monkey are refractory.	
	Avian cultures are moist, soft and readily form a homogeneous suspension.			

The rat is insusceptible to all three types of tubercle bacilli inoculated subcutaneously or given by the mouth; if a large dose is injected intraperitoneally the bacilli multiply abundantly in the organs and ultimately cause the death of the animal.

In the mouse, general tuberculosis may be produced by inoculation and by feeding with any of the types.

REFERENCES.

BANG, O., 1913, *Z. InfektKr.*, **13**, 215.
CALMETTE, A. & GUÉRIN, C., 1909, *C. R. Acad. Sci.*, Paris, **149**, 191.
CALMETTE, A., GUÉRIN, C. & GRYSEZ, V., 1913, *C.R. Soc. Biol.*, Paris, **74**, 310.
COBBETT, L., 1910, *J. Path. Bact.*, **14**, 563.

CUMMINS, S. L., 1924, *Tubercle*, Lond., **5**, 153.
GRIFFITH, A. S., 1907, *Royal Commission on Tuberculosis*, 2nd Interim Rep., Appendix **1**, 696.
GRIFFITH, A. S. & F., 1907, *Royal Commission on Tuberculosis*, 2nd Interim Rep., Appendix **3**, 235.
GRIFFITH, F., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **4**.
KÜSS, G., 1898, *De l'hérédité parasitaire de la tuberculose humaine*, Paris (Asselin & Houzeau).
LANGE, B., 1922, *Z. Hyg. InfektKr.*, **98**, 229; 1926, *Z. Tuberk.*, **46**, 455.
LANGE, B. & NOWOSELSKY, W., 1925, *Z. Hyg. InfektKr.*, **104**, 286.
PARROT, —., 1876, *C.R. Soc. Biol.*, Paris, **28**, 308.
TROMMSDORFF, R., 1909, *Arb. GesundhAmt.*, Berl., **32**, 568.
YERSIN, A., 1888, *Ann. Inst. Pasteur*, **2**, 245.

Tuberculosis in Man.

BY A. STANLEY GRIFFITH.

Tuberculosis in the human subject takes many forms. The visible lesions may be confined to one organ or gland, to one organ and its accompanying glands, or may be more or less widely disseminated over the body. Its characteristics and distribution vary with the age of the subject and the manner of invasion. In children and in susceptible adults the acute generalized forms predominate, while in more resistant individuals the lesions tend to remain localized in an organ or a gland and to run a very chronic course. When localized in one organ or gland the tuberculous process may become retrogressive and remain of limited extent, or it may be progressive and widely diffused throughout the organ. No part of the body is immune, but certain organs, such as the lungs in adults and the bronchial glands in children, are more frequently attacked than other structures.

INHERITED TUBERCULOSIS.

Before the demonstration by Villemin of the infectious nature of tuberculosis and the discovery by Koch of its specific agent, tuberculosis in the human being was generally believed to be an hereditary malady. This belief was founded on the frequency with which successive infants of tuberculous parents succumbed to the disease. But careful autopsies on fœtuses and infection experiments with their tissues [Grancher and Straus, Sanchez-Toledo, Vignal (quoted from Calmette, 1922)] have clearly demonstrated that tubercle bacilli with or without lesions are rare in the organs of the new-born offspring of tuberculous parents, and that hereditary transmission of tuberculosis is of minor importance in the propagation of tuberculosis in the human race.

Two possible modes of congenital transmission of tuberculosis have been differentiated: (*a*) conceptional or germinative and (*b*) transplacental.

(*a*) The former mode has never been demonstrated experimentally in animals or observed clinically in human beings and it is very improbable that it ever occurs. The growth of tubercle bacilli in the ovum would certainly result in its destruction. While experiments have shown that in the hen's egg tubercle bacilli artificially introduced can multiply and give rise to tuberculosis in the hatched chicken, this does not prove the possibility of germinative tuberculosis in man, since, as pointed out by Weber and Bofinger (1904), Koch and Rabinowitsch (1907) and others, infection of the hen's egg corresponds to placental infection.

(*b*) Transplacental infection of the human fœtus must be acknowledged as possible, but unquestionably it is very rare. In the bovine species this mode of transmission has been definitely established by the discovery in the new-born calf of well-developed tuberculosis involving primarily the liver and the portal glands. According to Bang (1905), however, congenital tuberculosis in the calf is of rare occurrence and is found only at a late stage of intrauterine life, when the uterus itself or the cotyledons are diseased.

The rarity of hereditary transmission of tuberculosis in the bovine species, as also in the human race, is well attested by the fact that the offspring, if separated immediately from their tuberculous parents and guarded subsequently against infection, generally remain perfectly free from the disease. A long latency of tubercle bacilli in human tissues, as claimed by von Baumgarten (1909), has not been proved.

POST-NATAL INFECTION.

As congenital tuberculosis is exceptional and a long latency of tubercle bacilli in human tissues without lesions has not been demonstrated, one may attribute practically every case of tuberculosis in children and adults to infection after birth.

Tubercle bacilli may enter the body through all surfaces which are open to invasion from the exterior. These are the skin, conjunctivæ and the lining membranes of the genito-urinary, alimentary and respiratory passages.

Skin. This mode of infection is relatively infrequent. It is uncertain whether tubercle bacilli can penetrate intact skin and pass into the adjacent glands, but there is no doubt that they can infect the skin directly through minute fissures, abrasions, or as a result of trauma. Primary tuberculosis of the skin usually takes one or other of two forms, namely, verruca tuberculosa (the post-mortem wart) and lupus vulgaris. The frequent origination of lupus vulgaris in the skin of the face, nose and neighbourhood of the anus and the higher proportion of bovine infections in lupus than in bone and joint tuberculosis are evidence in favour of direct infection of the skin.

Conjunctiva. It is very easy to produce cervical gland tuberculosis experimentally in animals by the instillation of tubercle bacilli into the conjunctival sac. Calmette (1922) believes that tuberculous glands in the neck may not infrequently be the result of a phthisical nurse wiping the eyes of an infant with a sputum-soiled handkerchief or by a phthisical person coughing infective particles into the eye. The proportion of the cases of human primary cervical gland tuberculosis produced in this way cannot be determined, since inhaled or ingested bacilli which are deposited on and pass through the nasal, buccal and pharyngeal mucous membranes affect the same groups of glands in the neck as are attacked as a result of conjunctival infection. It must, however, be very small, since in children under 5 years of age only about 15 per cent. of the cases of cervical gland tuberculosis are caused by human bacilli, and undoubtedly these in many instances must have gained entrance through the nose or mouth. Since in guiñea-pigs and monkeys tuberculosis of the conjunctiva and nasal passages frequently follows ocular instillation of tubercle bacilli, it is possible that in man these conditions may sometimes be caused by direct infection.

Genito-urinary. Primary infection through the genital and urinary tracts may conceivably occur, but is very exceptional.

Alimentary. When tubercle bacilli which have been ingested with food succeed in invading the body from the alimentary tract the entry is effected generally through the buccopharyngeal or the intestinal mucous membranes. The chief sites of entry in the upper digestive tract are the faucial, pharyngeal and lingual lymphoid follicles, and the first groups of glands to become affected are the upper cervical and retropharyngeal. Tubercle bacilli may also pass through the mucous membrane of the sockets of the teeth and thence into the submental glands. Tuberculous lesions in the tonsils are not infrequent though they are usually not conspicuous. Tubercle bacilli have been demonstrated by guinea-pig inoculation in hypertrophied tonsils when no lesions were visible to the naked eye. Pybus (1915) reported 6·7 per cent. of successful inoculations. Absorption from the intestinal canal takes place mainly through the agminated follicles and Peyer's patches; this is indicated in severe cases of primary intestinal tuberculosis by the increasing intensity of the tuberculous process as the ileo-coccal valve is approached and the diminishing number of lesions from the cæcum downwards.

The stomach is a rare portal of entry of tubercle bacilli in man.

Respiratory. This is the most frequent avenue of infection. Tubercle bacilli in inspired air may be deposited on the nasal, buccal or pharyngeal mucous membranes, and if absorbed affect the same groups of glands in the neck as ingested bacilli. The nose and mouth are not, however, efficient filters and arrest only the coarser particles. The careful experiments of many observers have shown quite conclusively that fine infective dust or droplets can readily enter the lungs with inspired air and penetrate to their most remote parts.

THE CHIEF PORTALS OF ENTRY IN HUMAN TUBERCULOSIS.

The classical inhalation experiments of Koch (1884), confirmed by numerous other investigators and supported by clinical and post-mortem observations, seemed to leave no room for doubt that the avenue of infection in human thoracic tuberculosis was the respiratory path, and that the vehicle of transmission was the dried or finely divided sputum of phthisical persons. For two decades after the discovery of the tubercle bacillus the doctrine that pulmonary tuberculosis is an inhalation disease was widely accepted and taught (Bulloch, 1909). Opinion was divided in the main as to the exact physical condition of the infective material, whether it was in the form of dust, as believed by Koch, or as moist droplets, as asserted by Flügge (1899) and his school. In 1903, von Behring in an address before the Naturforschung Versammlung at Cassel affirmed his belief in the alimentary origin of pulmonary tuberculosis. He was of the opinion that the infection was acquired in infancy or early childhood through the agency of cows' milk, the bacilli passing through the mucous membrane of the alimentary tract and glands in connection with it directly to the lungs. Behring presupposed both prolonged latency of tubercle bacilli and convertibility of the bovine into the human type in human tissues. Neither of these hypotheses has received confirmation. Calmette and Guérin (1905, 1906), Vallée (1906) and others supported Behring's theory of the alimentary origin of tuberculosis, which had been advanced by Chauveau as long ago as 1868, but disagreed that infection invariably occurred in infancy.

The teaching of Calmette at the present day is that the pulmonary tuberculosis of adults and the tracheo-bronchial gland tuberculosis of children are caused by bacilli which reach the lungs and glands by way of the lymph and blood-streams, that is to say, infection in these conditions is primarily lymphatic, and then haemal, and results chiefly from absorption of tubercle bacilli from the digestive tract.

There is not space to review the very numerous experiments which have been made to test the above hypothesis, and for a summary of these the reader should refer to the article by Bulloch in Allbutt and Rolleston's *System of Medicine* and to Cobbett's *Causes of Tuberculosis*. Briefly, these experiments have shown that air-borne tubercle bacilli, if in a sufficiently fine state of suspension, can readily pass directly into the most distant parts of the lung parenchyma and that it requires a much larger dose of bacilli to produce tuberculosis by ingestion than by inhalation.

Experiments, it is true, have shown also that tubercle bacilli may pass through intact pharyngeal and intestinal mucous membrane and, after massive doses, may infect distant organs, but in no susceptible animal, whether infected by feeding or by instillation into the eye of extremely small doses of bacilli, have lesions been found in the lungs or bronchial glands without co-existing lesions of equal or greater age in the mesenteric or pharyngeal glands. Thus, while the possibility of direct infection of the lung from the alimentary tract must be admitted, the experimental and bacteriological evidence indicates that this mode of origin of phthisis must be extremely infrequent. For instance, in Britain bovine tubercle bacilli are responsible for about 80 per cent. of the cases of cervical gland and primary abdominal tuberculosis in young children and, in view of the prevalence of bovine tuberculosis, must frequently enter the alimentary tract and be absorbed from it in small numbers, yet only one instance of primary tracheo-bronchial gland tuberculosis due to bovine bacilli has been recorded. All the numerous other instances of this form of tuberculosis were caused by human bacilli. In phthisis pulmonalis, also, bovine bacilli are of relatively infrequent occurrence.

Pathological Anatomy.

The picture of tuberculosis in the human subject varies with the age and susceptibility of the individual, the manner of infection and the virulence and dose of the bacilli. In infants and young children, in whom the primary localization and spread of tuberculosis can best be observed, the disease is characterized by lymphatic glandular enlargements and tendency to generalization. The anatomical distribution of the lesions seen in autopsies on tuberculous children generally corresponds to one or other of two well-defined forms, one in which the tracheo-bronchial glands, with or without pulmonary lesions, show greater enlargement and more extensive caseation than any other group of regional lymphatic glands, the other in which the glands of the alimentary tract (cervical or mesenteric) show the most advanced lesions.

Tracheo-bronchial gland tuberculosis is the most frequent, and in some countries and cities often the only form met with in young children. Many pathologists (Parrot, Ghon, Canti, &c.) are of the opinion, with which the writer concurs on the basis of his own observations, that tracheo-bronchial gland tuberculosis is almost always secondary to a focus in the lung; this focus may be so small as easily to escape detection, but is usually conspicuous and may contain a cavity. The bronchial glands are strictly regional lymphatic glands and those draining that part of the lung which contains the primary lesion are always found to be the most advanced in caseation.

The other distinct form of tuberculosis found at autopsies on children is that in which the mesenteric glands show the most advanced lesions, with or without intestinal ulceration. The bronchial glands in these instances are either not macroscopically tuberculous or show lesions at an earlier stage than those in the mesenteric glands. Primary intestinal and mesenteric gland tuberculosis varies in frequency in different countries. Statistics show that it is extremely rare in Vienna and New York, whereas in Scotland and England it is relatively common. Not infrequently one finds all three groups of regional glands enlarged and caseating, the condition sometimes appearing to be equally old in each group, but generally more advanced in the bronchial glands, this, in the writer's opinion, indicating simultaneous or contemporary infection through the digestive and respiratory tracts.

In infants and very young children tuberculous infection usually terminates in death from general miliary tuberculosis or tuberculous meningitis. Older children offer greater resistance, and the tuberculous process may be arrested in the glands or manifest itself in the chronic forms of tuberculosis with localizations in the bones and joints, skin, kidneys or testes. As the age of the child advances the disease begins more and more to resemble that usually found in the adult.

The characteristics of adult tuberculosis are its predominance in the lungs, the almost complete absence of caseous or even of calcified lesions in the tracheo-bronchial glands (though these glands always contain tubercle bacilli), and its slight tendency to generalization. The process does indeed extend, but usually by direct propagation within the lung and along continuous mucous surfaces (see below).

These differences in characteristics between the tuberculosis of children and adults are explained by Calmette (1922) in the following way. In children, not previously infected and highly susceptible, the tuberculosis is a primary manifestation and affects severely the lymphatic glands, because these in childhood easily retain and are suitable soil for the growth of the bacilli. Adult tuberculosis, on the other hand, is, he thinks, almost always a reinfection in individuals who possess a partial immunity in consequence of repeated (presumably minimal) infections. On this hypothesis, the characteristics of chronic pulmonary tuberculosis are due to a particular state of intolerance to the tubercle bacillus which characterizes antituberculous immunity ; it is, in other words, an allergic phenomenon of the same nature as that which is observed after reinfection of a tuberculous guinea-pig (phenomenon of Koch).

It seems to the writer, however, that the differences between child and adult tuberculosis may equally well be explained on the hypothesis that phthisis pulmonalis is a primary disease of the lungs in a naturally resistant individual. It is known from experiments that certain species of animals (the differential group, see page 169) become naturally more resistant as age advances without previous infections, and that primary affections of the lungs in animals often present the same phenomena of ulceration and excavation as those described in human beings. Moreover, in phthisis pulmonalis there is usually no anatomical evidence in the shape of caseous or calcareous changes in the glands such as might be expected if there had been previous infections in childhood.

MODES OF SPREAD AND DISTRIBUTION IN THE BODY.

Natural tuberculosis is at the outset always a local disease. Wherever in a susceptible host a virulent tubercle bacillus finds lodgment the histological changes characteristic of tuberculosis are initiated. This primary focus may extend locally, and may also be the source of origin of secondary tuberculous deposits in various parts of the body. Local extension is often considerable both in the lungs and in the mucous membrane of the alimentary tract. In the lungs, whether the tuberculous process starts in a bronchiole, an infundibulum or an alveolus, a well-defined lesion is formed which quickly undergoes central necrosis and caseation. The caseous mass then softens, ulcerates into a bronchus and is discharged in the sputum. The cavity thus formed increases in size by progressive infiltration and caseation of the surrounding tissues. The caseous lining of such a cavity always shows abundant tubercle bacilli, and is an excellent medium for the growth of tubercle bacilli as well as of other organisms.

If the focus is in a lymphoid follicle or plaque in the intestines, the nodule which forms soon becomes necrotic and caseous in the centre, then softens and ulcerates. The ulcer, at first circular or oval, has a tendency to spread circumferentially and become ring-like. In contrast to a typhoid ulcer a tuberculous ulcer rarely perforates.

The distribution of tuberculosis from the primary focus (or from secondary foci) takes place generally in one or other of three ways : (*a*) by the lymph-stream, (*b*) by the blood-stream, (*c*) along continuous surfaces.

(*a*) Distribution by the lymphatics is the earliest method of spread and occurs more readily in children than in adults. The bacilli, either free or intracellular, are transported by the lymph flowing along the afferent vessels to the lymphatic glands. At any point along these vessels bacilli may become stationary and give rise to tubercles. One often sees an eruption or a chain of little tubercles in the lymphatic vessels in the neighbourhood of an intestinal ulcer, along the vessels of the mesentery and in the lungs in the peribronchial tissues. Tubercles also form in the lymphatic glands which often attain greater dimensions than the primary focus. From one set of glands tubercle bacilli pass to other groups or directly into the thoracic duct, which may itself become the seat of lesions. In this way bacilli, in large or small numbers, gain access to the blood-stream and cause acute miliary or chronic disseminated tuberculosis.

(*b*) The blood-stream may be infected not only through the thoracic duct, but also directly by an irruption into a vein, or an artery, of tubercle bacilli from a focus which has invaded and ulcerated the wall of the vessel. Miliary tubercles may be profuse in all the chief organs and of equal size, indicating a simultaneous invasion of numerous tubercle bacilli. More often the tubercles vary in size from the just visible grey granulation to a yellow caseous nodule, indicating successive invasions, and in distribution according to the vessel invaded and the organ. Although tubercle bacilli may circulate with the blood all over the body, certain organs remain free when others are closely beset with tubercles, indicating that different organs differ widely in susceptibility to tuberculosis. If only few bacilli are disseminated by the blood- or lymph-stream, the more chronic forms of tuberculosis are produced with localizations in the bones, or joints, or kidneys, &c.

(*c*) Spread by continuity of surface is most frequently seen in ulcerative pulmonary tuberculosis. The breaking down of a focus in the lung or the rupture of a caseous gland into a bronchus with the consequent production of infective sputum may give rise through aspiration to tuberculous broncho-pneumonia, by expectoration to tuberculous ulceration of the trachea, larynx, tonsils and pharynx, nose and skin of the face, and by swallowing the sputum to tuberculous ulceration of the gut and to lupus of the buttocks.

Tuberculosis of the kidneys may lead to a descending infection of the ureters and bladder, or infection may ascend to the kidneys from a tuberculous testis or epididymis. In the mamma also infection may spread from the acini down the teats.

Extension to serous surfaces takes place from an adjacent focus. The pleura and pericardium may be infected directly from a lung focus, or the peribronchial glands, and the peritoneum from an intestinal ulcer, the Fallopian tubes, abdominal lymphatic glands or a subcapsular tubercle in the liver or spleen. Serous surface tuberculosis may be localized and fibrinous, ending in adhesions, or widespread and miliary. Pleural tuberculosis may be accompanied by a serous or purulent exudate.

REFERENCES.

Anderson, C. W., 1921, *Arch. Derm. Syph.*, Wien, Orig., **129**, 26.

Bang, B., 1905, *7th Internat. Vet. Congress*, Budapest.

von Baumgarten, 1909, *Deuts. med. Wschr.*, **2**, 1729.

de Besche, A., 1913, *Deuts. med. Wschr.*, **39**, 452.

Blacklock, J. W. S., 1929, *Ann. Rep. Med. Res. Coun. for 1927–8.*

Bulloch, W., 1909, Allbutt's *System of Medicine*, London, **5**, 299; 1911, Horace Dobell Lecture, Roy. Coll. Physicians.

Burnet, E., 1912, *Ann. Inst. Pasteur*, **26**, 868.

Calmette, A., 1922, *L'infection bacillaire et la tuberculose chez l'homme et chez les animaux*, Paris, Masson et Cie.

Calmette, A. & Guérin, C., 1905, *Ann. Inst. Pasteur*, **19**, 601; 1906, *ibid.*, **20**, 353 and 609.

Canti, R. G., 1919, *Quart. J. Med.*, **13**, 71.

Cobbett, L., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **1**.

Eastwood, A., Griffith, F. & Griffith, A. S., 1914, *Rep. Loc. Govt. Bd. Publ. Hlth.*, n.s., No. **88**.

Eber, A., 1913, *Zbl. Bakt.*, Abt. I, Orig., **70**, 229.

Edington, J. W. & Guest, D., 1926, *J. Hyg.*, Camb., **25**, 109.

Flügge, C., 1899, *Z. Hyg. InfektKr.*, **30**, 107.

Fraser, J., 1912, *J. Exp. Med.*, **16**, 432.

Gaffky, 1907, *Tuberculose*, Berlin, **6**, 437.

Ghon, A., 1912, *Der primäre Lungenherd bei der Tuberkulose der Kinder*, Berlin.

Gosling, R. & Montanus, J., 1924, *J. Med. Res.*, **44**, 513.

Griffith, A. S., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **1**; 1914, *Brit. Med. J.*, i, 1171; 1916, *J. Path. Bact.*, **21**, 54; 1928, *ibid.*, **31**, 875.

Hart, C. & Rabinowitsch, L., 1917, *Z. Tuberk.*, **27**, 334.

Kirchner, M., 1922, *Z. Hyg. InfektKr.*, **98**, 447.

Kitasato, S., 1909, *Z. Hyg. InfektKr.*, **63**, 517.

Koch, M. & Rabinowitsch, L., 1907, *Virchows Arch.*, **190**, 246.

Koch, R., 1884, *The Aetiology of Tuberculosis*, New. Syd. Soc., **115**, 63.

Lindemann, E. A., 1913, *Arb. GesundhAmt.*, Berl., **45**, 197.

Mitchell, A. P., 1914, *Brit. Med. J.*, i, 125.

Möllers, B., 1928, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, **5**, 615.

Munro, W. T., 1928^{1}, *Lancet*, Lond., i, 384; 1928^{2}, *Proc. R. Soc. Med.*, **22**, 1206.

Munro, W. T. & Cumming, W. M., 1926, *Edinb. Med. J.*, **33**, 97.

Neufeld, F., Dold, H. & Lindemann, E. A., 1912, *Zbl. Bakt.*, Abt. I, Orig., **65**, 467.

Park, W. H. & Krumwiede, C., Jr., 1912, *J. Med. Res.*, **27**, 109.

Pybus, 1915, Hunterian Lecture, *Lancet*, Lond., i, 1009.

Rothe, 1911, *Veröff. Koch.-Stift.*, Heft. **2**, 1.

Rothe & Bierrotte, 1913, *Veröff. Koch.-Stift.*, Heft. **8-9**, 87.

Ungermann, 1912, *Tuberk. Arb.*, Berl., **12**, 109.

Vallée, H., 1906, *C.R. Acad. Sci.*, Paris, **142**, 1101.

Wang, C. Y., 1916, *J. Path. Bact.*, **21**, 14; 1917, *ibid.*, **21**, 131.

Weber & Bofinger, 1904, *Tuberk. Arb.*, Berl., **1**, 83.

The Incidence in Human Tuberculosis of the different Types of Tubercle Bacilli and the Stability of Type.

By A. Stanley Griffith.

Incidence.

At the British Congress on Tuberculosis held in London in 1901, Koch made the memorable announcement that human tuberculosis was distinct from bovine tuberculosis and could not be transmitted to cattle. This conclusion was based on his experimental work, and, so far as it relates to the more common variety of human tuberculosis, has since been confirmed. But Koch went further than this and argued from the results of his investigations that the bacillus of bovine tuberculosis was not dangerous for man. This assumption aroused great controversy. It was pointed out that while it might be true that the human tubercle bacillus was incapable of causing progressive tuberculosis in calves and swine, it did not necessarily follow that the bovine tubercle bacillus must be harmless for man. Koch's opinion was based chiefly on his observation, from autopsies on children in Berlin, that cases of primary intestinal tuberculosis were rare in Germany. Such was not the case in this country, however, and for this reason McFadyean, Woodhead and others contested Koch's assertion that bovine tuberculosis was not a danger to man.

It was obvious after Koch's pronouncement that the matter could not remain in doubt, and in England a Royal Commission was appointed to inquire into the relations of human and animal tuberculosis, and official investigations were also instituted in other countries. The Commission reported in 1911, and the work was subsequently continued by the medical officers of the Commission.

The investigations in this country and abroad have been very extensive and have proved conclusively that Koch was wrong in assuming that bovine tubercle bacilli were a negligible factor in human tuberculosis. Space does not permit a detailed review of these researches which are briefly summarized below.

British Investigations.

The types of tubercle bacilli have been determined (by Cobbett, Eastwood, and A. S. and F. Griffith) in a total of 1,441 cases, of which 1,321 were derived from England and Wales (Table II) and 120 from Scotland (Table III).

The relatively high proportion of autopsies in which the bovine tubercle bacillus was found is attributable to the fact that in two of the four component series the material was selected, preference having been given to those cases in which the portal of entry appeared to be the alimentary canal. In the two other series, examined by A. Eastwood and F. Griffith (1914) and A. S. Griffith (1914) respectively, the material investigated was obtained from an unselected series of children dying from all causes under the age of 13 years. The total number of children examined was

223, of whom 133 showed evidence of tuberculous infection, and 113 yielded cultures of tubercle bacilli. In 94 of the 113 cases the cultures were of the human type, and in 18 they were of the bovine type ; in one instance both types of bacilli were present. Thus, bovine bacilli were found in 16·8 per cent. of the cases. In the children under 5, the percentage bovine was 21·3 (13 out of 61) and in the children over 5 it was 11·5 (6 out of 52).

TABLE II.

English Statistics.

	Number of Cases.	0–5 Years.			5–15 Years.			15 Years and Over.			Percentage Bovine (All Ages).
		Hum.	Bov.	Per cent. Bov.	Hum.	Bov.	Per cent. Bov.	Hum.	Bov.	Per cent. Bov.	
Cervical glands ..	116	3	18	*85·7*	28	26	*48·1*	32	9	*21·9*	*45·7*
Lupus	176	23	36	*61·0*	48	51	*51·5*	15	3	*16·6*	*51·1*
Scrofuloderma ..	54	7	6	*46·1*	17	10	*37·0*	13	1	*7·1*	*31·5*
Bone and joint ..	511	60	24	*28·6*	277	65	*19·0*	81	4	*4·7*	*18·2*
Genito-urinary ..	23	—	—	—	2	1	*33·3*	17	3	*15·0*	*17·4*
Pulmonary ..	202	—	—	—	10	—	*0·0*	190	2	*1·0*	*1·0*
Tuberculous meningitis	33	2	1	*33·3*	13	7	*35·0*	9	1	*10·0*	*27·3*
Autopsies ..	183	72	29	*30·1a*	48	7	*14·3b*	21	1	*12·5c*	*23·0*
Miscellaneous ..	23	2	1	*33·3*	10	1	*9·1*	9	—	*0·0*	*8·7*
Total	1,321		—			—			—		—

(*a*) Including 2 mixed infections (H and B).
(*b*) ,, 1 ,, infection ,,
(*c*) ,, 2 ,, infections ,,

In 125 of the 183 autopsies the anatomical evidence was definitely in favour of the respiratory tract as being the portal, or one of the portals of entry of the infecting organism, and in 124 of these, or 99·2 per cent., the type of tubercle bacillus found was human. In 39 cases the primary manifestations of the disease were in the mesenteric glands or intestines ; of these 33, or 84·6 per cent., were infected with bovine tubercle bacilli.

Only two other English series have been published. Bulloch and Fildes (1911) examined the sputum of 29 persons with phthisis pulmonalis and obtained tubercle bacilli of the human type only. Edington and Guest (1926) isolated cultures from 38 cases of bone and joint tuberculosis in Sheffield, and found the bovine bacillus in two instances (5·2 per cent.).

In Scotland, a number of other workers have investigated the type of tubercle bacillus in human tuberculosis. Mitchell (1914) examined 72 cases of cervical gland tuberculosis in children up to 12 years of age, and found bovine bacilli in 65, or 90·3 per cent. Fraser (1912) examined 70 cases of bone and joint tuberculosis, 67 in children under 12 years of age and 3 in adults. He reported that 41, or 61·2 per cent., of the children were infected with bovine bacilli, 23 with human bacilli and 3 with both types. Wang (1917) obtained cultures from 88 cases of tuberculosis of various kinds. He found bovine bacilli in 11 of 20 children under 16 years of age, or 55·0 per cent., and in 7 of 68 adults, or 10·3 per cent. Of 10 alimentary cases, 9 or 90 per cent. yielded cultures of bovine type. All the above cases were derived from Edinburgh and district. Munro and Cumming (1926) examined 55 cases of surgical tuberculosis from other parts of Scotland and found bovine bacilli in 20 instances, or 36·4 per cent. Blacklock (1929) obtained cultures of tubercle bacilli from 84 cases of fatal tuberculosis in Glasgow. The results showed that 80·0 per cent. of primary abdominal tuberculosis were due to the bovine type of bacillus, while 96·0 per cent. of primary pulmonary tuberculosis were due to the human type.

TABLE III.

Scottish Statistics (A. S. Griffith).

	Number of Cases.	0–5 Years.			5–15 Years.			15 Years and Over.			Percentage Bovine (All Ages).
		Hum.	Bov.	Per cent. Bov.	Hum.	Bov.	Per cent. Bov.	Hum.	Bov.	Per cent. Bov.	
Cervical glands ..	17	1	3	*75·0*	3	7	*70·0*	1	2	*66·6*	*70·6*
Bone and joint ..	30	12	6	*33·3*	8	2	*25·0*	2	—	*0·0*	*26·7*
Pulmonary ..	73	—	—	—	—	—	—	72	1	*1·4*	*1·4*
Total	120	—			—			—			—

The most notable result of the investigations in Scotland has been the discovery of bovine tubercle bacilli in the sputum of a comparatively large number of persons suffering from phthisis pulmonalis. So far 13 cases have been recorded in Scotland, 5 of which occurred in a series of 70 cases examined by Munro (1929), while only 2 out of 231 cases were found in England. The possibility of the transmission of bovine tubercle bacilli from man to man through the agency of sputum must, therefore, be admitted. Whether the apparently higher proportional incidence of bovine infections in human tuberculosis in Scotland than in England may be attributed to transmission of bovine bacilli from two sources, namely, phthisical human beings and tuberculous cattle, is matter for further inquiry, and it is desirable that this should be undertaken.

Foreign Statistics.

Möllers (1928) has published a table summarizing the types of tubercle bacilli found in cases of human tuberculosis by various investigators. His statistics are given in Table IV in a simpler form, from which the English and Scotch figures included by him are omitted.

TABLE IV.

	Total Number.	Human.	Bovine.	Mixed.	Percentage Bovine.
Cervical gland ..	219	166	53	—	24·2
Bone and joint ..	149	145	4	—	2·7
Genito-urinary ..	39	38	1	—	2·6
Lupus	122	97	24	1	20·5
Meningitis	58	54	4	—	6·9
Autopsies	526	440	86	—	16·3
Sputum	1,020	1,017	1	3	0·4

Of individual foreign investigations, the following from different countries are separately mentioned for comparison with the English statistics.

In Germany, Gaffky (1907) and Rothe (1911) examined in unselected series 400 children who had died from various causes, and found tuberculous infection in 78. In 75 of these cases the infection was due to the human tubercle bacillus, and in 1 to the bovine bacillus; in the remaining 2 they were unable to obtain cultures, and, therefore, the type of bacillus was not determined. Ungermann (1912) investigated a similar series of 171 children (0 to 12 years), and found tuberculous infection in 39 cases. Of these 37 were due to human, and 2 to bovine bacilli. Rothe and Bierotte (1913) found bovine bacilli in 5 out of 37 cases of lupus. Kirchner (1922), on the other hand, considered that 12 of the 23 strains which he had obtained from lupus cases belonged to the bovine type. It is possible that in Germany during the war years there was a temporary increase in the incidence of bovine tuberculosis on human beings. Hart and Rabinowitsch (1917), for example, reported that cases of primary intestinal and mesenteric gland tuberculosis had increased from an average of 0·8 per cent. of the autopsies in 1910–14 to 2·6 per cent. in 1915–16; they obtained cultures from 6 cases and stated that 5 of the 6 strains were bovine.

In Norway, de Besche (1913) examined a series of 134 dead children and found tuberculosis in 52. Cultures of tubercle bacilli were obtained from 50 cases, and of these 47 were human, 2 were bovine and 1 was a mixture of human and bovine. In Copenhagen, Anderson (1921) examined 23 cases of lupus and found human bacilli in 19 and bovine bacilli in 4 of the cases. In Paris, Burnet (1912) obtained human bacilli only from

59 cases of external tuberculosis. Park and Krumwiede (1912) reported from America that of 56 cases of general tuberculosis, 7 were due to bovine bacilli and 1 was infected with both types. Gosling and Montanus (1924) isolated 48 strains of tubercle bacilli from spinal fluids in tuberculous meningitis and found that 3, or 6·3 per cent., were bovine. In Japan, Kitasato (1909) examined 152 cases, mainly adults, and found tubercle bacilli of the human type only.

From the results of these different investigations, one is justified in concluding that human tuberculosis of bovine origin is proportionately more frequent in the British Isles than in any other country of the world.

Summary and Conclusions.

1. Human tuberculosis may be the result of infection either with the human or with the bovine type of tubercle bacillus. The avian type plays no part in the causation of human tuberculosis in Great Britain.
2. The relative proportion of the human and bovine types of bacilli varies in the different clinical and anatomical-pathological forms of the disease and at different age periods.
3. The human tubercle bacillus is the more frequent cause of human tuberculosis and was found in 83·2 per cent. of an unselected series of tuberculous children dying from all causes under the age of 13 years. The study of the anatomical distribution of the tuberculous lesions in children infected with human bacilli has shown that the primary lesions were intrathoracic in 82·0 per cent. of the cases ; in some of these there was evidence also of contemporary infection through the mucous membrane of the alimentary tract.
4. The bovine tubercle bacillus plays a part in the causation of all the chief varieties and anatomical forms of human tuberculosis, and is responsible for a considerable amount of mortality and disease, especially in children. In an unselected series of tuberculous children dying from all causes under the age of 13 years, this type of bacillus was found in 16·8 per cent. of the cases. About 70 per cent. of the children infected with bovine bacilli died of tuberculosis, the great majority from general tuberculosis or tuberculous meningitis. The distribution of the lesions found at autopsies on children infected with bovine bacilli pointed clearly to the alimentary tract as the usual portal of entry of the bacilli. In one case only was the anatomical evidence in favour of the respiratory tract as the portal of entry.
5. Of the various clinical forms of the disease, lupus and cervical gland tuberculosis have yielded the highest proportion of bovine infections. Next in order comes scrofuloderma. Then follow those clinical forms of the disease, namely, tuberculous meningitis, bone and joint tuberculosis and genito-urinary tuberculosis, which are caused by blood-borne tubercle bacilli.
6. The proportion of bovine infections in all forms of tuberculosis is highest in children under 5 years of age. At later age periods there is a

progressive decline. The number of instances of bovine infection in adolescents and adults is, however, not inconsiderable. In cervical gland tuberculosis, for example, 25·0 per cent. of the persons over 15 years of age were infected with bovine tubercle bacilli.

7. The age incidence of bovine infections, taken in conjunction with the anatomical situation of the primary lesions, points to cows' milk as the source of the infection with bovine tubercle bacilli.

8. Bovine tuberculosis is an important source of human tuberculosis in Great Britain. The collected statistics for the different countries show that human tuberculosis of bovine origin is proportionately more frequent in Great Britain than in European countries or in North America. The relative incidence of bovine infection is higher in Scotland than in England and Wales. In Scotland, a larger proportion of cases of phthisis pulmonalis due to bovine tubercle bacilli have been discovered than in England.

THE STABILITY OF TYPE OF TUBERCLE BACILLI IN THE ANIMAL BODY.

Under experimental conditions. Numerous experimental investigations have been made in this country and abroad with the object of ascertaining whether the characters and properties which distinguish the different types of tubercle bacilli one from another change under the influence of animal tissues different from their natural environment. Thus, human tubercle bacilli have been made to reside for long periods in the tissues of the ox, the goat and the rabbit ; bovine bacilli in the dog, the chimpanzee and the monkey ; avian bacilli in mammals and mammalian bacilli in birds ; the tubercle bacilli of warm-blooded animals in cold-blooded animals ; while in some experiments the bacilli were enclosed in collodion capsules which were inserted in the peritoneal cavities of animals with a view to subjecting them to the action of tissue fluids which might permeate the walls of the capsules.

The results of these experiments have been diverse. In the vast majority the bacilli remained entirely unaltered, although the duration of residence was in many instances very prolonged. In a small minority of experiments the strain recovered was of a different type from that with which the experiment was begun. This change has been interpreted by some observers as evidence that tubercle bacilli can sometimes adapt themselves to thrive in the tissues of an animal for which ordinarily they are not pathogenic, and in so doing take on the characteristic properties of the bacilli which naturally produce tuberculosis in that species of animal.

The experiments which were made to control and repeat these results have given ground for the belief that the recorded changes of type were not mutations but due either to the occurrence of spontaneous tuberculosis or to the use of a mixed virus at the outset of the experiment.

The Royal Commission on Tuberculosis, whose work on this subject was very comprehensive, proved that certain instances of apparent transformation of human into bovine bacilli, which occurred in the early part of their work, were due to the presence in the original material of bovine as well as of human bacilli.

Mixed infection in human tuberculosis is not rare—in the English series there were 5 instances, and Möllers (1928) has collected altogether 17, including the English examples—and may be the explanation, as supposed by Neufeld, Dold and Lindemann (1912), of some of the positive results obtained by Eber (1906–13). The more common source of error, however, in modification experiments is the occurrence in one of the passage animals of spontaneous infection with a type of bacillus different from that inoculated. The investigators (Cobbett and A. S. and F. Griffith), who did the practical work of the Commission, came to the conclusion that tubercle bacilli are very stable and cannot be transformed by passing the bacilli through the bodies of different species of animals.

Cobbett (1917) has critically reviewed the experiments of the Commission as well as those of foreign observers and they need not be considered in detail. Several experiments, however, are particularly noteworthy on account of their long duration and the evidence they give of the high degree of stability possessed by the different types of bacilli. These are summarized in Table V.

TABLE V.

Type of Bacillus.	Species of Animal.	Number of Passages.	Duration of Experiment.	Result.
Human ..	Cow	Single	852 days	No change.
	Calf (3 expts.) ..	2 to 5	506 to 512 „	„
	Rabbit ..	Single	725 „	„
	Fowl (2 expts.)	5	643 and 685 „	„
	Rat	8	1,614 „	„
Bovine ..	Chimpanzee ..	5	542 days	No change.
	Dog	10	1,122 „	„
	Fowl	3	475 „	„
	Toad	7	8 years	„
Avian	Goat	Single	3 years	No change.
	Rabbit and guinea-pig	7	763 days	„
	Toad	Single	2½ years	„

Stability of type under natural conditions. The question arises whether tubercle bacilli which under natural conditions succeed in establishing themselves in a fresh host of a different species may acquire new characters and become indistinguishable from the type most commonly found in that species. May, for example, bovine bacilli change into human bacilli during residence in human tissues ? It is conceivable that a change of type may take place either abruptly, in which event there would be no evidence of the origin of the infection, or gradually, the process being marked by a number of forms exhibiting intermediate characters.

A complete transformation of bovine into human bacilli in the human body, if it ever occurs, must be extremely infrequent, since the strains obtained from the great majority of cases of human tuberculosis originating in the alimentary tract, which is practically the only channel of entry available for bovine bacilli, correspond exactly to, or vary only slightly from, bacilli of bovine type. The assumption that, since bovine bacilli are of relatively frequent occurrence in children and rare in adults, conversion of type has occurred is not valid, as adult pulmonary tuberculosis is with rare exceptions clearly a disease of respiratory origin, due to the human type of bacillus, the infection being transmitted from man to man. Moreover, in a few instances bovine bacilli typical in every respect, or only slightly reduced in virulence, have been obtained from the sputa of phthisical persons, and not infrequently from other chronic forms of tuberculosis in adolescents and adults, for example, lupus and bone and joint tuberculosis. The duration of the tuberculosis in some of these patients was very prolonged and yet the bovine bacilli had preserved their distinctive features. In one case of lupus of thirty years' duration the culture obtained was a fully virulent, dysgonic bovine strain. In another lupus case of fifty-two years' duration the bacilli were typically bovine culturally and in virulence, only the virulence was not quite up to standard.

These examples, taken from a large number of similar observations, are equivalent to modification experiments in the human being and show that bovine bacilli can retain their distinctive features for an indefinite period in human tissues. The only modification which bovine bacilli appear to undergo during the progress of a natural infection either in man or in the horse or the pig is in pathogenicity, which in certain circumstances becomes decreased for the usual experimental animals. It is noteworthy that cultural characteristics in these instances remained unchanged, and are therefore a more stable property than virulence.

In the section on virulence (p. 225) attention is called to the occurrence in human tuberculous lesions of strains which do not conform exactly either to the human or to the bovine types of tubercle bacilli. Some writers regard strains which diverge in certain respects from the standard types as being intermediate between bovine and human tubercle bacilli. This the author considers an erroneous view. There are three atypical forms of tubercle bacilli found in human beings which might, by those who uphold the doctrine of unity of tubercle bacilli, be regarded as intermediate between bovine and human bacilli ; these are designated dysgonic human, eugonic bovine and attenuated bovine strains. The following are reasons for not regarding them as intermediate or transitional forms.

Dysgonic human strains. These resemble bovine strains in nothing except in growing with difficulty on glycerin-agar, potato and broth ; on serum and egg media they grow like eugonic human bacilli and for guinea-pigs and rabbits they exhibit the virulence of the human type of bacillus. They are not increased in virulence or altered in cultural characters by passage through the rabbit, but can in some instances be converted into typical human bacilli by growth on glycerin media.

Eugonic bovine strains. These have been found in the pig as well as in man, and, therefore, one cannot regard the eugonic character of the strains found in human beings as necessarily due to the influence of human tissues.

Attenuated bovine strains. In respect of virulence for calves and rabbits these viruses occupy an intermediate position between bovine and human bacilli. Had the pathogenic powers of the viruses been lowered for the calf and rabbit alone, and not for the monkey and guinea-pig as well, the conclusion would have been inevitable that in these instances we were dealing with cultures which were becoming transformed. But inoculation experiments on monkeys and guinea-pigs, animals equally susceptible to both types of bacilli, have shown that the viruses were reduced in virulence for these species also ; in fact, the nearer their virulence for bovines and rabbits approached that of the human bacillus, the lower it proved for the monkey and guinea-pig. Thus, the apparent bridging of one gap was accompanied by disclosure of another equally broad. In the writer's opinion the atypical strains of tubercle bacilli found in human tuberculosis are merely variants of the bovine or of the human type and are not intermediate between the bovine and human types of bacilli.

REFERENCES : see p. 190.

Avian Tuberculosis.

BY A. STANLEY GRIFFITH.

In 1882 Robert Koch found bacilli with the morphological and staining peculiarities of the tubercle bacillus in the tumour-like lesions of fowls which previously Paulicki (1872) had suggested were probably of the same nature as ' perlsucht ' in cattle and miliary or caseous tuberculosis in human beings. Koch at first regarded the tuberculosis of birds as identical with that of mammals and for some time this view was generally accepted. After a while, however, the inquiries of many observers, more particularly those of Nocard and Roux (1887), Rivolta (1889) and Maffucci (1892) showed that the organism causing tuberculosis in birds differed, not only in cultural characteristics but also in virulence, from that causing the disease in mammals. Koch subsequently (1890), when opportunity was afforded him of examining cultures of the bacilli, which previously had not been possible on account of lack of fresh material, confirmed these observations and concluded that the organism of avian tuberculosis was distinct from that of mammalian tuberculosis. Weber and Bofinger (1904) and others came to a like conclusion. On the other hand, Cadiot, Gilbert and Roger (1891 to 1898), M. Koch and L. Rabinowitsch (1907) and others on various grounds regarded the avian bacillus as merely a variety of the tubercle bacillus adapted to grow in the tissues of the fowl.

Whether one regards the avian bacillus as a variety of the tubercle bacillus or as a distinct species, there is no doubt that the organism which is commonly found in the tuberculous lesions of fowls has well-defined characters and properties which distinguish it clearly from tubercle bacilli of mammalian tuberculosis (see p. 289).

NATURAL TUBERCULOSIS OF AVIAN ORIGIN.

Under domestic conditions avian tuberculosis is fostered and spread by poultry kept in crowded and insanitary yards or pens. In the domestic fowl tuberculosis is almost always of alimentary origin and is brought about by the ingestion of food contaminated with the droppings from infected fowls. When, as is commonly the case, there are tuberculous ulcers in the intestines of the fowls, the fæces are apt to contain tubercle bacilli in very large numbers. Tuberculosis in fowls may also be acquired by scavenging the carcasses of fowls which have died of the disease. Infection through the air passages may occur in fowls which are made to scratch for their food in dry litter in covered sheds. Tubercle bacilli occasionally enter the body through fissures or abrasions about the mouth or in the skin of the feet. The avian virus may be conveyed from infected to healthy poultry through the agency of sparrows, mice and rats. Avian tuberculosis occasionally appears enzootically among fowls and pigeons, causing enormous losses.

Avian bacilli have been demonstrated in the eggs of infected birds, and hereditary transmission, while probably uncommon, is also a source of infection which should be considered and guarded against.

In captivity in Zoological Gardens tuberculosis has been recorded in practically every avian species—in waterfowl and birds of prey, as well as in graminivorous birds. Under domestic conditions birds vary widely in receptivity. Of the common domesticated species, fowls, turkeys, pheasants and pea- and guinea-fowls are the most frequently attacked. Pigeons are less susceptible. Geese and ducks, even when living in close association with infected poultry, commonly escape tuberculosis. The reason for this is not clear since these birds are certainly susceptible.

Among wild birds living free tuberculosis is exceptional. Instances have been recorded in a sparrow, a kestrel, a partridge and a lapwing.

DISTRIBUTION AND CHARACTERS OF THE LESIONS IN NATURALLY TUBERCULOUS BIRDS.

Natural tuberculosis in birds is, as a rule, a very chronic process and characterized by the formation in the organs, especially the abdominal viscera, of conspicuous tumour-like nodules. Koch and Rabinowitsch (1907) have recorded the frequency with which different organs were tuberculous in a series of 116 birds of various species which died in the Zoological Gardens, Berlin, and Van Es and Schalk (1914) have summarized the results of various observers who studied the distribution of tuberculous lesions in domestic fowls. The different series show some variation in the percentage incidence of tuberculosis on the different organs, but all are in agreement that the liver and the spleen are more frequently attacked than any other organ.

In the domestic fowl, as previously mentioned, infection is usually of alimentary origin and lesions can generally be found at the portals of entry of the bacilli. Any part of the alimentary canal from the mouth to the cloaca may show tuberculous changes, but the seats of election are the neighbourhood of the cæca, the Peyer's patches and the lymphoid tissue of the proventriculus.

Intestinal lesions usually take the form of nodules which project from the serous surface of the gut. They vary in size, commonly ranging from a millet seed to a pea; in advanced cases they may attain the dimensions of a walnut, and occasionally even larger tumours have been seen. The large nodules are yellowish white externally and are often lobulated, covered with shaggy villous processes and adherent to adjacent structures; sometimes they nearly completely encircle the gut.

Intestinal nodules may be of two kinds. One form is solid, composed throughout of dense caseo-necrotic substance, and originates in the lymphatic vessels or tissues in the outer part of the intestinal wall; over these nodules the mucous membrane of the gut is seldom ulcerated. The other form of nodule on section is hollow and ulcerous and communicates with the intestinal canal by a wide or narrow fistulous opening. The walls are of fibrous tissue lined internally with caseo-necrotic substance, the ulcerated surface of which is greenish or brownish black and often has fæces adherent to it.

These ulcerated saccular dilatations are produced when the tuberculous process begins in the Peyer's patches, which is the usual event in experimental infections. The stages in their formation are as follows: the lymphoid tissue of the patch becomes hypertrophied and indurated; caseous foci appear which unite and ulcerate through the mucous membrane; gradually, owing to infiltration and weakening of the wall and pressure of the intestinal contents, the patch begins to bulge outward; the bulging is progressive but remains localized and through contraction of the surrounding muscles becomes constricted at the line of junction with the gut.

In addition to the large nodules there may be numerous small caseous nodules, some subserous, others submucous. Tubercles and irregular caseous nodules are often seen also in the mesentery.

A third form of tuberculosis of the intestine is a diffuse enteritis, caused, in the writer's opinion, by blood-borne bacilli, since it has occurred in birds after intravenous inoculation of avian bacilli. The small intestine, especially the duodenum, is thickened; there is a round-cell infiltration of the submucosa and numerous tubercle bacilli in and on the mucous membrane, which may be eroded.

The liver and spleen in fowls with generalized tuberculosis are almost invariably affected. In acute cases they are enlarged and closely beset with grey granulations or caseating tubercles of uniform size. In chronic cases the lesions may vary in size from a millet seed to a walnut. The large nodules are very hard and shell out readily from the soft liver tissue ; on section they are composed of a yellowish, waxy, hyaline, caseous substance—the ' masse vitreuse ' of French authors—often showing dark concentric rings. Central softening may occur but is rare ; so also is calcification. The liver in chronic cases is enlarged, pale yellow, fatty, and very soft. Both the liver and the spleen are liable to hæmorrhages, which may rupture the capsule and cause death.

The lungs are often attacked, but less frequently than the liver and spleen. The lesions are in the form of discrete tubercles or isolated irregular caseous nodules or masses. Pulmonary tuberculosis may be primary and the lesion is then often of considerable extent.

The bone-marrow is frequently the seat of lesions, which may lead to spontaneous fractures of the long bones. Cobbett (1917) mentions one case in which the bone-marrow throughout the skeleton was largely transformed into cheesy caseous substance. Tuberculosis of the joints is less frequent than in the bones and is usually secondary to disease in the latter. The joints become distended with mucinous pus containing caseous flakes.

There are no lymphatic glands in the fowl, but in the lower cervical region on each side there is a chain of glandular bodies which are often affected with tuberculosis. These bodies are very useful in diagnosis since the existence of tuberculosis can often be detected by examining them during the life of the fowl or in the carcass after death. Other organs—kidneys, ovaries, testes, pleura, air-sacs, heart and skin—are rarely affected.

Cutaneous tuberculosis, while rare in domestic fowls, is comparatively frequent in parrots when these are kept as pets. The lesions, in the form of warty, scaly or horny excrescences of a greyish colour, are seen most commonly on the skin of the head, namely, around the eyes, root of bill and on the skull, and only occasionally on the wings and legs. The tuberculosis is usually confined to the skin but may be disseminated, when it is said to affect the lungs principally. The cutaneous form of tuberculosis in the parrot is caused by human tubercle bacilli and is primary, the bacilli gaining access to the skin tissues through scratches and abrasions. In zoological gardens parrots may be infected casually with avian bacilli and in these circumstances they exhibit the abdominal type of tuberculosis.

Experimentally, parrots are susceptible to all three types of bacilli. According to F. Griffith (1911) the bovine bacillus is the most, and the avian bacillus the least, virulent of the three types. Other species of birds are highly resistant to experimental infection with human and bovine bacilli, and, although epidemics of tuberculosis in the poultry yard have frequently been attributed to infection from human or bovine sources, there are no authenticated instances of natural infection in domestic fowls either with human or bovine bacilli. On the other hand, Koch and Rabinowitsch (1907) state that they found human bacilli in three captive wild birds, namely, two eagles and a jay.

There are characteristic differences between the tuberculous lesions of birds and those of mammals. In birds the necrotic lesions produced by the avian bacillus are dense, hyaline and exhibit little tendency to caseous softening or calcareous degeneration. In avian lesions tubercle bacilli are more abundant and more frequently intracellular than mammalian bacilli in mammalian tuberculosis. Avian lesions seldom show true giant cells and contain a greater amount of connective tissue than mammalian lesions. According to Eastwood (1911) one of the essential differences between the avian and the mammalian bacillus is that the former exerts a less powerful irritant or stimulant action upon the endothelial cell.

NATURAL TUBERCULOSIS OF AVIAN ORIGIN IN ANIMALS.

Among the domesticated species of mammals infection of avian origin is most frequent in swine. In England about 30 per cent. of the cases of localized tuberculosis in swine were caused by avian tubercle bacilli ; in America and on the Continent the proportion is sometimes much higher. Isolated examples of spontaneous avian tuberculosis have been recorded in the horse by M'Fadyean (1918), in the sheep by A. S. Griffith (1925), in the rabbit by Cobbett (1913) and A. S. Griffith (1928[1]), in the mouse and rat by Weber and Bofinger (1904), Koch and Rabinowitsch (1907), and in the bovine animal by A. S. Griffith (1911) and Plum (1926). The observations of Plum have not been confirmed. He states that he examined six cows with tuberculous abortion disease and found avian tubercle bacilli in the uterus, placenta and uterine exudates ; he thinks that the gravid uterus of cows is a favourable nidus for avian bacilli.

Among captive wild animals cases of spontaneous avian tuberculosis are of rare occurrence. A. S. Griffith (1928) obtained avian bacilli from a case of general tuberculosis in a kangaroo. N. Lucas (1925) isolated avian tubercle bacilli from the mesenteric gland of a kangaroo rat. L. Rabinowitsch (1906) reported the isolation of avian bacilli from a monkey.

Human beings are considered by some continental authorities to be susceptible to infection with avian tubercle bacilli. Löwenstein (1913) claims to have isolated avian bacilli from the sputum of a woman as well as from two cases of renal and one case of skin tuberculosis, and is of the opinion that acute tuberculous septicæmia (' Typhobacillose Landouzy ') is also ascribable to infection with tubercle bacilli of avian type. Lipschutz (1914) believes that this type of bacillus is of frequent occurrence in human skin tuberculosis. Rabinowitsch (1907) reported the isolation of avian bacilli from the spleen of a man who died of miliary tuberculosis. Jancsó and Elfer (1910) state that they found avian bacilli in a mesenteric gland of a girl eight years old.

In view of the high resistance of apes and monkeys to experimental infection with avian bacilli it is questionable whether human beings can develop true tuberculosis after infection with avian bacilli. The evidence from English and Scottish investigations support this opinion, since none of the strains obtained from upwards of 2,000 tuberculous human beings belonged to this type.

REFERENCES.

CADIOT, GILBERT & ROGER, 1891, *Congrès pour l'étude de la tuberculose*, 69, Paris ; 1891 to 1898, several papers, *C.R. Soc. Biol.*, Paris.

COBBETT, L., 1913, *J. Comp. Path.*, **26**, 33 ; 1917, *The causes of tuberculosis*, Cambridge (Univ. Press).

EASTWOOD, A., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **5**, 1.

GRIFFITH, A. S., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **1**, 374B and 386 ; 1925, *J. Comp. Path.*, **38**, 157 ; 1928[1], *ibid.*, **41**, 53 and 109 ; 1928[2], *J. Hyg.*, Camb., **28**, 198.

GRIFFITH, F. 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **4**, 167.

JANCSÓ, N. & ELFER, A., 1910, *Beitr. klin. Tuberk.*, **18**, 175.

KOCH, M. & RABINOWITSCH, L., 1907, *Virchows Arch.*, **190**, 246.

KOCH, R., 1884, *The Ætiology of Tuberculosis* (trans. 1886), New Sydenham Society, **115**, 65 ; 1890, *Zbl. Bakt.*, **8**, 563.

LIPSCHUTZ, B., 1914, *Arch. Dermat. Syph.*, Wien, Orig., **120**, 387.

LÖWENSTEIN, E., 1913, *Wien. klin. Wschr.*, **26**, 785.

LUCAS, N., 1925, *J. Path. Bact.*, **28**, 123.

M'FADYEAN, J., 1918, *J. Comp. Path.*, **31**, 225.

MAFFUCCI, A., 1892, *Z. Hyg. InfektKr.*, **11**, 445.

NOCARD & ROUX, 1887, *Ann. Inst. Pasteur*, **1**, 19.

PAULICKI, 1872, *Beitr. vergl. Anat.*, Berlin.

PLUM, N., 1926, *Cornell Vet.*, **16**, 250.

RABINOWITSCH, L., 1906, *Deuts. med.Wschr.*, **32**, 866.

RIVOLTA, 1889, *Jber. Fortschr. pathog. Mikroorg.*, 313.

STRAUS, I. & GAMALÉÏA, N., 1891, *Congrès pour l'étude de la tuberculose*, 66, and *Arch. de méd. exp.*, **3**, 457.

VAN ES, L. & SCHALK, A. F., 1914, *Bull. N. Dak. Agric. Expt. Sta.*, No. 108.

WEBER & BOFINGER, 1904, *TuberkArb.*, Berl., **1**, 83.

Tuberculosis in Animals.

BY A. STANLEY GRIFFITH.

TUBERCULOSIS IN DOMESTICATED SPECIES OF ANIMALS.

Under conditions of domestic life all species of animals are subject to tuberculosis. The different species, however, vary widely in their susceptibility ; cattle, pigs and fowls, for example, frequently contract the disease, while sheep and goats, on the other hand, are seldom affected. Three types of tubercle bacilli are concerned, namely the bovine, avian and human types.

Cattle.

Frequency. Of all the domesticated species of mammals bovine animals are most subject to tuberculosis and are responsible for the greater part of the tuberculosis of other mammals. Bovine tuberculosis is very widespread and exists in all countries where cattle are bred for food. The incidence of the disease varies in the different countries and is always highest in stalled cattle. It is mainly a disease of adult life. The proportion of calves found tuberculous in slaughter-houses generally ranges from 1 to 4 per cent., and in adult stalled animals from about 30 to 70 or 80 and even to 100 per cent.

Pathological anatomy. Natural tuberculosis in cattle is generally of a chronic, slowly progressive nature ; only occasionally it takes the acute form similar to that which follows experimental inoculation of large doses of bacilli. Any tissue in the body may be the seat of tuberculous lesions, and the disease may be localized in the lymphatic glands or confined to one organ or to one cavity or generally disseminated. The lymphatic glands are the most frequently involved and are often the only tissues to show lesions. The different organs of the body are attacked with a variable frequency. From the figures published by Ostertag (1903), it would appear that the lungs are more often the seat of tuberculous lesions than the abdominal organs, and the disease is more often localized in the thoracic than in the abdominal cavity. In the calf, tuberculosis is more frequently of alimentary origin than in adult bovines and affects predominantly the lymphatic glands. Congenital tuberculosis is not uncommon, and in this condition the most advanced lesions are found in the liver and hepatic glands.

The pulmonary lesions of cattle show great variability in number, dimensions and appearance. Usually the lungs contain many masses of varying size, those just under the pleura, which at these points is thickened and covered with tuberculous growths, projecting as rounded bosses. On section the tumours show a fibrous wall enclosing bright yellow caseous substance, which may be dense and gritty, or of a mortary consistency or broken down into a thick yellow viscous pus. Often there are cavities communicating with bronchi. Owing to the anatomical peculiarity of the bovine lung these cavities are limited in extent by the thickened interlobular septa and do not, as in human beings, become multilocular. The pulmonary tissue around the masses may be normally pink and crepitant or there may be a surrounding zone of hepatized tissue ; sometimes owing to plugging of a bronchus the whole or the greater part of a lobe may become solid and airless. One meets also with irregular patches of caseous pneumonia, in which there may be cavities enclosing a fœtid pus. Sometimes the lungs contain nodules resembling sarcomata, or nodules which are completely fibrous (Vallée and Panisset, 1920).

The thoracic glands are generally affected when there are lesions in the lungs ; they may, however, be tuberculous without obvious pulmonary lesions. The glandular lesions take many forms and are found in various stages of development ; they may be discrete, encapsulated, caseous and softened, or quite calcareous; or they may be extensive and transform the whole gland into yellow fibro-caseo-calcareous substance which in places may be softened. Sometimes the glands are merely hyperplastic.

The pleura often shows lesions which have a quite peculiar and characteristic appearance ; this is the so-called ‘ grapes ’, ‘ pommelière ’ or ' perlsucht ' disease. In the most advanced stage the lesions cover the greater part of the parietal and visceral pleura and are in the form of firm fleshy, pinkish, translucent or greyish white grape-like masses flattened out by pressure and connected to the lung or the parietes by pedicles which are either short or much drawn out. The lesions begin in the lymphatic fringes of the margins of the lungs and of the ribs and around the tendons of the diaphragm as minute grey granulations which subsequently coalesce. The pleural tuberculosis may exist without accompanying pulmonary lesions. Lesions of the same type occur on the pericardium, where they may form layers several centimetres thick, and on the omentum and parietal and visceral peritoneum.

Next to the lungs the organ most frequently the seat of tuberculous lesions is the liver, then the spleen, and lastly the kidneys.

Tuberculosis of the mamma is of relatively frequent occurrence, particularly in cows with advanced generalized tuberculosis. The disease may involve one quarter only or may be diffused throughout several quarters. The affected quarter is enlarged, indurated and nodular and on section shows a fibrous infiltration and caseous and calcareous foci. The milk of a tuberculous udder always contains tubercle bacilli in large numbers, and the condition is a dangerous source of infection to human beings.

Bacteriology. The chronic slowly progressing lesions of natural tuberculosis in the bovine animal are always caused by virulent tubercle bacilli of the bovine type. It is possible that in some situations, e.g. the skin, or the joints, the bovine bacillus may undergo modification in virulence similar to that which human (and bovine) tubercle bacilli undergo in human tissues, but no systematic observations bearing on this point have been made.

Human and avian tubercle bacilli, which under experimental conditions are incapable of producing progressive tuberculosis in the bovine animal, have rarely been found in naturally acquired tuberculosis. The author (Griffith, 1913) found human tubercle bacilli in a mesenteric gland lesion of a calf born of a cow which had been vaccinated with living human tubercle bacilli; he also (Griffith, 1911) isolated an avian strain from a calcareous nodule in the mesenteric glands of a calf which had been the subject of an experimental infection with human tubercle bacilli.

Swine.

Frequency. Next to cattle, pigs are the most subject to natural tuberculosis. The proportion found tuberculous in different countries and districts varies widely, and is greatest where swine are fed largely with unsterilized skimmed milk and milk residues, or ingest the fæces of cattle. Tuberculosis is sometimes very frequent among pigs which never come into contact with cattle, directly or indirectly, and in these instances it has been shown that the infection has been derived from tuberculous poultry.

Pathological anatomy. Natural tuberculosis in swine arises almost exclusively through infection from the alimentary canal. The intestines may show ulcers of the mucous membrane or tubercles and nodules in their walls, but more often no lesions are visible to the naked eye. The tonsils and the lymphoid follicles of the pharynx and tongue show visible tuberculosis, in the form of ulcers and caseous nodules, more frequently than the intestines. The most advanced lesions are found in the glands along the alimentary canal, all of which may be attacked, but those of the neck more frequently than those of the abdomen. The disease when localized is usually limited to the glands situated near the angle of the jaw. The extent of the disease in the glands shows wide variation, from a few small tubercles to great enlargement and uniform caseo-calcareous change. In the strictly localized cases the glands are often not at all enlarged and contain a varying number of small caseo-calcareous tubercles, usually discrete, which can readily be picked out from the gland substance. When enlarged, the glands may show part or the whole of their substance replaced either by dense firm grey or pinkish grey translucent tissue infiltrated with a yellowish white network gritty from calcification, or in more advanced cases by caseo-calcareous mortary material surrounded by a fibrous capsule. The softening process begins around the periphery of the necrotic substance, which at this stage can be shelled out *en masse.* The enlarged neck glands—the scrofula of the ancients—may be seen during life, and they may ulcerate through and infect the skin.

In the young pig infected with bovine bacilli generalization of the disease is rapid and progressive. The lungs exhibit a varying number of discrete caseo-calcareous tubercles and nodules ; sometimes there are patches of caseous pneumonia. The liver contains miliary tubercles and nodules. The spleen often contains nodules varying in size, sometimes as large as a Barcelona nut ; they are sharply circumscribed and frequently form greyish white prominent bosses, sometimes with central depression ; on section they show fibrous walls with caseo-calcareous centres. The kidneys are less frequently affected and may contain wedge-shaped areas. The parietal and visceral pleura may show pinkish, flattened mushroomed growths of a similar nature to the ' perlsucht ' disease of cattle. The omentum may contain discrete tubercles and nodules and the peritoneum may show an eruption of tubercles. The mamma is often affected in old sows, but some authorities consider that it is not so frequently the seat of tuberculosis as in cows ; the tuberculous lesions are generally discrete caseo-calcareous nodules. All the glands of the body may be affected, particularly the bronchial, portal, inguinal and prescapular glands. Tuberculosis is comparatively common in the bones—particularly those of the vertebral column and the ribs and joints—and has been described in the brain, meninges, muscles, connective tissues, eye, ovaries and adipose tissue ; in the three last regions it is exceptional.

Bacteriology. Natural tuberculosis in the pig may be caused by any one of the three types of tubercle bacilli. The relative proportion of these three types naturally depends on the degree of exposure to tuberculous cattle, poultry and human beings respectively. The bovine bacillus is responsible for practically all the instances of severe local and obvious general tuberculosis. The human bacillus has been found only in swine in which the tuberculous lesions—in the form of discrete tubercles—were strictly localized to the submaxillary glands. Avian bacilli may cause quite extensive caseo-calcareous changes in the submaxillary and mesenteric glands, but are usually responsible for discrete tubercles without any tendency to spread. Avian bacilli do not, however, remain restricted to the glands ; they are often disseminated over the body of the pig and may cause small lesions in the liver, lungs and attached glands (Eastwood and F. Griffith, 1914), or conspicuous nodules in the spleen (A. S. Griffith, 1928[1]), or severe generalized tuberculosis (de Jong, 1910).

Horse.

Frequency. Tuberculosis in the horse is not rare in this country, but there are no statistics relating to its frequency. In France, cases of the disease have been exceptional ; out of 62,500 horses slaughtered in a Paris abattoir only 4 were found to be tuberculous. German statistics show a higher proportion ; in recent years it was in the neighbourhood of 0·19 per cent.

Pathological anatomy. Tuberculosis in the horse is of great interest on account of its peculiar pathological features. Nocard (1896) divided equine tuberculosis into two groups, abdominal and thoracic forms. The abdominal form is the more frequent and is characterized by the development in the mesenteric and retroperitoneal glands, in the spleen, and sometimes in the liver, of irregular tumour-like lesions which often attain enormous dimensions. These tumours are generally of homogeneous consistency, dense and greyish white throughout, or they may contain caseo-purulent foci which are often extremely rich in tubercle bacilli. Sometimes the walls of the intestines contain numerous dense greyish white nodules ranging in size up to that of a walnut, and ulcers of various sizes may be seen in the mucous membrane, particularly in the Peyer's patches. In one case (M'Fadyean, 1918) there were lesions in the intestines resembling the chronic hypertrophic enteritis of bovines (Johne's disease). Nodules may also be found on the pleura and pericardium accompanied by large effusions into the serous cavities. Miliary tuberculosis of the lungs is often a terminal event of the abdominal form. Thoracic tuberculosis is, however, occasionally primary. The lungs in these instances may contain nodules and masses similar to those in the abdominal organs or they may be diffusely infiltrated with dense sarcoma-like masses which do not provoke inflammatory reaction of the pulmonary tissues. The thoracic glands are enlarged and enclose miliary tubercles or dense fibro-caseous nodules.

The bones, especially the vertebral, may be the seat of tuberculous changes. The mucous membranes of the nose, trachea and bronchi sometimes show ulcers. Lesions are of rare occurrence in other regions.

Bacteriology. The earliest investigations of the ætiology of equine tuberculosis were made by Nocard (1894). His first culture, which was derived from a case of tuberculosis localized in the lung, had mammalian characteristics. Subsequently Nocard (1896) investigated specimens from two horses attacked with the abdominal form of the disease. He inoculated guinea-pigs and rabbits and sowed a large number of glycerin potato tubes thickly with mesenteric gland juice which was extraordinarily rich in tubercle bacilli. On one tube only he obtained a culture which was vigorous and abundant and exhibited in subculture the characteristics of the avian type of bacillus. The culture caused acute tuberculosis in two rabbits and a fowl inoculated intravenously. A guinea-pig inoculated subcutaneously with the culture and killed 29 days later showed local tuberculosis only. Two guinea-pigs inoculated subcutaneously with a little spleen pulp of one of the horses died of atypical general tuberculosis, a result which is never produced by typical avian tubercle bacilli. Nocard admitted that the lesions provoked by this equine virus were not absolutely identical with those caused by the avian bacillus, and he attributed the difference in pathological activity to modification undergone by the bacillus as a result of its acclimatization in the organism of the horse. In any case he considered it certain that the bacillus approached much more closely the bacillus of avian tuberculosis than the bacillus of mammalian tuberculosis.

Nocard's statement that the abdominal form of equine tuberculosis is caused by the avian tubercle bacillus has not been confirmed. It is true that a typical avian strain was obtained by M'Fadyean (1918) from the intestines of a horse, but in this animal the gross lesions, which were limited to the intestines, had none of the characteristic naked-eye features of ordinary tuberculosis in the horse and were at first thought to be caused by Johne's bacillus. A condition of the intestines similar to that observed by M'Fadyean has never been produced in any horse fed with pure cultures of the avian tubercle bacillus (F. Griffith, 1911 ; Titze, 1907).

All the instances of the typical form of abdominal tuberculosis in the horse investigated by other observers, both in this country and abroad, have yielded tubercle bacilli of mammalian type only. The cultures obtained by F. Griffith (1911), M'Fadyean (1924), A. S. Griffith (1928[1]), Zwick and Zeller (1913) and Stableforth (1929), from 45 cases of typical equine tuberculosis, the majority of which showed tuberculomatous masses in the abdominal viscera, all exhibited the cultural characteristics of the bovine type. These equine strains, though typically bovine in respect of their cultural characters, exhibited a wide range of variation in their pathogenic properties. Some were as virulent as bovine bacilli derived directly from bovine tuberculous lesions ; others were not more virulent for calves and rabbits than the human tubercle bacillus, while the remainder possessed an intermediate degree of virulence. It is possible that Möllers (1912) who attributed to human bacilli one case of equine tuberculosis may have been mistaken in his diagnosis and may have classified as human a strain which was in reality a modified bovine strain. Passage through calves and rabbits (F. Griffith, 1911) of two equine strains with low experimental virulence resulted in these strains acquiring the full virulence of the bovine type. This instability and the gradation of virulence of equine strains, taken in conjunction with the fact that modified strains do not occur in the ox, from which the horse undoubtedly derives its infection, show conclusively that bovine tubercle bacilli become modified in pathogenic properties during the progress of the disease in the horse.

Sheep.

Frequency. Tuberculosis is a rare disease among sheep in this country. This is evident from the statistics compiled by Hayhurst (personal communication). Of 255,000 sheep slaughtered at Blackburn in 1910–14, only 2 were found to be tuberculous. The proportion was even less at the Metropolitan Cattle Market Abattoir, only 3 out of 1,069,163 showing lesions which macroscopically resembled tuberculosis. Fullerton (1902) has reported one case and M'Fadyean (1900 and 1902) two cases of tuberculosis in the sheep. On the other hand, tuberculosis of sheep is not rare in continental countries and M'Fadyean thinks that the greater frequency there is due to the fact that in most of these countries the sheep are often for long periods of the year kept in houses.

Pathological anatomy. The tuberculous lesions of the sheep bear a close resemblance to those of the bovine animal. The pleura may show 'perlsucht' growths. In Fullerton's case the lungs were extensively consolidated and beset with numerous tubercles. In one of the author's two cases there were fibro-caseo-calcareous nodules in the lungs, liver and spleen, and all the lymphatic glands were much enlarged and highly calcified. In the second case the mesenteric glands were calcareo-caseous throughout, and the organs contained gritty miliary caseous tubercles.

Bacteriology. In the two cases published by M'Fadyean there is little doubt that the tuberculosis was caused by tubercle bacilli of the bovine type. In two cases described by the author (Griffith, 1925), the bacilli causing the tuberculosis were of the avian type. One of the latter strains was, however, atypical in cultural characters. These results show that the sheep under natural conditions is susceptible to casual infection with avian as well as with bovine tubercle bacilli.

Goat.

Frequency. Tuberculosis is exceedingly rare among goats in this country. On the other hand, in continental countries, where they run in flocks and in close association with cattle, naturally acquired tuberculosis in goats is relatively frequent.

Pathological anatomy. Tuberculosis in the goat is usually generalized and involves the lungs more severely than other organs. The pulmonary lesions tend to break down and form cavities. Mammary tuberculosis is not uncommon, and in such circumstances the milk may be a source of infection to human beings.

Bacteriology. The infection is probably always derived from tuberculous cattle, since, under experimental conditions, human and avian tubercle bacilli are incapable of producing progressive tuberculosis in the goat. One case examined by the author (Griffith, 1917) yielded tubercle bacilli of the bovine type.

Cat.

Frequency. Tuberculosis in cats is comparatively frequent. In continental countries the proportion of cats found tuberculous has varied from 2 up to 18 per cent.

Pathological anatomy. Tuberculosis in the cat is generally of alimentary origin, as is indicated by the fact that the glands of the neck and mesentery usually show the most advanced lesions. Sometimes these glands are very greatly enlarged and extensively caseous and softened. Those in the neck may ulcerate through the skin, and the mesenteric glands may rupture into the peritoneal cavity causing diffuse tuberculous peritonitis. In other cases the glands are only moderately enlarged and contain caseo-calcareous areas. The pharyngeal and intestinal mucous membranes rarely show lesions.

The organs of the cat most frequently attacked are the lungs. Pulmonary tuberculosis takes several forms. Sometimes the lungs are beset with tubercles or nodules ; or they may contain and be largely replaced by firm grey translucent sarcoma-like areas enclosing purulent or caseous foci ; sometimes they are riddled with cavities which communicate with bronchi, or which ulcerate into the pleural cavity, leading to acute tuberculous pleurisy. The bronchial glands are hypertrophied and show small caseous areas. Tuberculosis of the lungs is sometimes primary, and in such cases the bronchial glands are greatly enlarged and caseous.

Of the abdominal organs the spleen shows tuberculous lesions more frequently than the liver or the kidneys. The eyes, bones, areolar tissues and skin may be the seat of lesions. Cutaneous tuberculosis occurs not infrequently and, like the ulcerative pulmonary form, is a very dangerous manifestation, since the purulent discharges contain tubercle bacilli in great abundance.

Bacteriology. Rabinowitsch-Kempner (1921) examined tuberculous material from 5 cats and reported that 3 were infected with human tubercle bacilli and 2 with bovine tubercle bacilli. The statement that natural tuberculosis in the cat may be caused by human tubercle bacilli is not supported by the experimental work of the Royal Commission on Tuberculosis (1907 and 1911), or by the bacteriological investigations carried out by F. Griffith (1911), Cobbett (1926) and A. S. Griffith (1925). These authors obtained cultures from 20 tuberculous cats, and all the strains proved to be of the typical bovine type.

Dog.

Frequency. There are no statistics relating to the frequency of tuberculosis in the dog in this country, but there is little doubt that it is not of common occurrence. In France, and especially in Paris, tuberculosis is comparatively frequent among dogs.

Pathological anatomy. Tuberculosis in the dog is often confined to the thorax or to the abdominal cavity and is then discovered only after the death of the animal from some other cause. The disease may, however, be widespread and severe and cause death.

Lesions are found more often in the lungs than in other organs and take the form either of caseous broncho-pneumonia, disseminated miliary or nodular tuberculosis, or tuberculous masses having a sarcomatous appearance, or ulcerative tuberculosis with large single or numerous small cavities, the pus of which contains tubercle bacilli in great number. According to some authorities tuberculosis in the dog is mainly a pulmonary disease. This is confirmed by Cobbett (1917), who analysed the findings of several authors in 71 dogs and stated that pulmonary disease was present in 75 per cent.

The serous surfaces, more particularly of the pleura and pericardium, show lesions with relative frequency. The tuberculosis takes several different forms, viz. acute sero-fibrinous or suppurative, grey granulomatous plaques or irregular thickenings and large sarcoma-like masses filling up the mediastinal spaces. Pericarditis may occur alone but is usually associated with pleurisy. The heart-muscle itself is sometimes attacked.

In the abdominal form the mesenteric glands are enlarged, caseous and softened, the caseo-pus containing large numbers of tubercle bacilli. Of the abdominal organs the liver is the most frequently affected. The kidneys are sometimes found tuberculous, the spleen rarely. In alimentary cases tuberculous lesions are rarely seen in the mucous membrane of the alimentary tract. Cutaneous tuberculosis is said by Petit (1900) and others not to be rare.

Bacteriology. Of 4 cases examined in this country 3 (Griffith, 1928[1]) yielded human and 1 (Wilson and Lovell, 1928) bovine tubercle bacilli. Schornagel (1914) found human bacilli in 6 cases and bovine bacilli in 2 cases. Rabinowitsch-Kempner (1921) isolated human bacilli from 18 cases and bovine bacilli from 1. Malm (1912), on the other hand, found only bovine bacilli in 5 cases. It will thus be seen that the naturally acquired tuberculosis of the dog may be caused either by human or by bovine tubercle bacilli.

Rabbit.

The domesticated varieties are remarkably free from tuberculosis. They may contract bovine tuberculosis if fed with cows' milk for the purpose of promoting growth and improving the quality of the fur and wool, or avian tuberculosis if allowed to run over the same ground as infected poultry (Cobbett, 1913), or either type of disease when living in the same house as experimentally tuberculous animals.

Guinea-pig.

The guinea-pig, kept under good conditions and protected from the obvious sources of infection, is rarely the subject of spontaneous tuberculosis. Like the rabbit, the guinea-pig may contract the disease when living in the same house as experimentally tuberculous animals and the infection may be of respiratory or alimentary origin. The tubercle bacilli obtained from the lesions of spontaneously affected guinea-pigs are generally of the bovine type, though human and avian tubercle bacilli have been found in a few instances.

English Statistics.

TABLE VI.

Types of Tubercle Bacilli in Domesticated Animals.

Species of Animal.	Number of Cases.	Types of Tubercle Bacilli found.		
		Bovine.	Human.	Avian.
Horse	37	36	—	1
Pig	258	183*	6†	74‡
Cat	20	20	—	—
Dog	4	1	3	—
Goat	1	1	—	—
Sheep	4	2	—	2
Cattle	52	50	1	1
Guinea-pig	10	6	2	2
Rabbit	8	4	—	4
Fowl	13	—	—	13

* Including 5 cases infected with two types.
† Including 1 in which bovine bacilli were also present.
‡ Including 4 in which bovine bacilli were also present.

TUBERCULOSIS IN WILD ANIMALS.

Tuberculosis in warm-blooded animals living wholly in the wild state is unknown ; at least no authenticated example of such occurrence has been put on record. A few instances in wild animals living free have been mentioned by Herbert Fox (1923), but in these the possibility that they had acquired the infection directly or indirectly through association with human beings or domesticated animals could not be excluded.

When wild animals are kept in captivity, as in zoological gardens and other institutions, tuberculosis is very common among them and is one of the chief causes of mortality. This is a consequence of the conditions under which they are kept. Except in the most modern zoological gardens the animals are confined to restricted quarters and are often herded together in small cages. In these circumstances, tuberculosis in one animal readily spreads to other occupants of the cage and the disease may assume the character of an epidemic (Scott, 1927). The infection is transmitted from tuberculous members of the public or from tuberculous attendants by coughing, spitting, &c., or it may be conveyed to them in food contaminated with tubercle bacilli. Infection may also spread from one group of animals to another through the agency of animals such as mice and sparrows living free in the menagerie or gardens.

Of the mammalian orders the primates generally furnish the largest number of cases. All species of apes and monkeys are highly susceptible and readily contract tuberculosis in confinement.

The type of infecting bacillus has been determined in a large number of spontaneously tuberculous monkeys. F. Griffith (1911) obtained cultures of human type from a chimpanzee with general tuberculosis of alimentary origin and from a rhesus monkey with chronic pulmonary and slight disseminated tuberculosis. Cobbett (1917) cultivated human tubercle bacilli from two lemurs. The author (Griffith, 1928[2]) investigated 28 cases of natural tuberculosis in monkeys, including 3 apes, and found that 8 were infected with bovine bacilli and 20 with human bacilli. The proportion of bovine infections in the latter series is much higher than in the series examined by Rabinowitsch (1906). She obtained cultures from 33 monkeys which had died in the Zoological Gardens, Berlin, and found that 25 were of human type, 3 were of bovine type, 1 was avian, while 3 exhibited intermediate characters; from the remaining monkey 2 types were obtained, human from the lung and bovine from the spleen.

Tuberculosis in the spontaneously infected monkey generally takes either of two forms. In one the bacilli entering the body by way of the alimentary tract produce ulcers in the intestines, enlargement and caseation of the glands (neck and abdominal) of the alimentary tract and general tuberculosis. In this form the bronchial glands show lesions which are less extensive and in an earlier stage than those in the mesenteric glands. The resemblance of this form of tuberculosis to that in children resulting from alimentary infection is very close.

In the other form the primary lesions are intrathoracic and the bronchial glands are generally enlarged and caseous; the mesenteric glands may show lesions, but they are less extensive than in the bronchial glands. It is usual to find one or more lobes of the lung in a condition of caseous consolidation, with cavitation in long-standing cases. This form of disease is, in the writer's opinion, due to air-borne infection.

The respiratory form of tuberculosis in monkeys, as in human beings, is usually caused by human tubercle bacilli. It is noteworthy, therefore, that in a series of 28 monkeys examined by the writer (Griffith, 1928[2]), 4 with this form of tuberculosis were infected with bovine tubercle bacilli. Spontaneous tuberculosis in the monkey, as these investigations show, may be caused either by human or by bovine tubercle bacilli. To these two types of bacilli, monkeys of all species are equally highly susceptible, and infection with either type, whether natural or experimental, is always followed by generalization of the disease and progression to a fatal termination. There is no feature of the disease in monkeys which differentiates one infection from the other.

The mammalian order, next to the primates, with the highest incidence of tuberculosis is that of the ungulata. The fact that tuberculosis in members of this order is generally more severe in the lungs than in any other organ and is often confined to the thorax, points to the respiratory tract as being the usual channel of entry of the bacilli. Fox (1923) also came to this conclusion from a study of the distribution of the lesions in 33 cases.

The tuberculous lesions in the different species of wild ruminants are similar in character to those found in the nearly related domesticated species. In the wild and domestic Bovidæ fibrosis and calcification of the lesions are common features. In deer and antelopes the pulmonary lesions, like those in the domestic goat, often break down and form cavities.

A rhinoceros which died at the Zoological Society's Gardens, London, showed miliary tuberculosis of the lungs and nodules on the pleura and surface of the liver ; the type of infecting bacillus proved to be bovine.

The elephant is susceptible to tuberculosis and two instances have been reported (Dammann and Stedefeder, 1909 ; Thieringer, 1911). Both animals had pulmonary tuberculosis and in one some of the vertebral bodies were the seat of disease. Cultures of tubercle bacilli were obtained and proved in each instance to be of the human type.

In the ungulata as in the primates the human and bovine types of tubercle bacilli only have been obtained from the tuberculous lesions, but in the former the bovine tubercle bacillus has occurred the more frequently. Human tubercle bacilli were found in a gnu, an antelope, a blesbok and a peccary (Griffith, 1911 and 1928[2]). The gnu (*Gorgon taurinus*) died of general tuberculosis, the disease being of great severity in the lungs, which were very fibroid and packed with caseous nodules. The antelope (*Oryx beisa*) showed extensive tuberculosis of the lungs, which were riddled with cavities. The blesbok (*Damaliscus albifrons*) had miliary and nodular tuberculosis of the lungs. It is interesting that the human tubercle bacillus, which is incapable of producing progressive tuberculosis in domestic cattle, goats and pigs, can cause tuberculosis in the wild species of ruminants equal in severity to that set up by the bovine type of bacillus.

Tuberculosis is not rare among carnivorous animals in captivity, and has been described in the lion, tiger and other members of the family Felidae and also in the bear, the fox, jackal, the genet and the coati. Except in the jackal, genet and coati the type of infection does not appear to have been determined, but it is not unlikely, since these species of animals are usually fed with uncooked offal, that the infecting bacilli were bovine. This was the type of bacillus obtained from the genet (*Genetta pardina*) and the jackal (*Canis cancrivorous*) ; the tuberculosis in the genet was, however, confined to the thorax and there was no anatomical evidence of infection having occurred by way of the alimentary tract. Two cases of tuberculosis in the coati (*Nasua narica*) yielded strains of human type. In the tuberculous coati the disease is of the fibro-caseous variety and usually involves the serous surfaces, particularly those of the peritoneal cavity.

Members of other orders of placental mammals in captivity in zoological gardens rarely contract tuberculosis. The order Chiroptera is represented by one animal, an Indian fruit bat, which died with severe thoracic tuberculosis. It was infected with tubercle bacilli of bovine type which were extremely abundant in the lesions.

Marsupial animals are susceptible to tuberculosis and several instances (about a dozen) have been described in the Proceedings of the Zoological Society (Scott, 1928). Two cases of abdominal tuberculosis in pet opossums have recently been recorded by Herbert Fox (1928). In two kangaroo-rats and one kangaroo from the Zoo, the type of bacillus was determined. Lucas (1925) obtained from the mesenteric gland of one of the kangaroo-rats a strain of avian tubercle bacilli. The rat died six weeks after arrival in the Gardens and at autopsy showed an enlarged purulent mesenteric gland, containing large numbers of acid-fast bacilli, and soft white tubercles in the liver. Griffith (1928[2]) isolated from the other kangaroo-rat, which died of severe thoracic tuberculosis, a culture of tubercle bacilli of the bovine type. They were extremely abundant in the lesions. The kangaroo (*Macropus fuliginosus*) died of severe and widespread glandular tuberculosis and tuberculosis of the shoulder joint. Acid-fast bacilli were present in enormous numbers in the pus from the glands and the joint. The bacillus was of the avian type.

The results of bacteriological investigations show that tuberculosis in captive wild mammals may be caused by any one of the three types of tubercle bacilli—bovine, human or avian (see Table VII).

TABLE VII.

Types of Tubercle Bacilli in Captive Wild Mammals.

English Statistics.

Order.	Number of Cases of Tuberculosis.	Types of Tubercle Bacilli obtained.		
		Bovine.	Human.	Avian.
Primates ..	30	8	22	0
Lemuridæ ..	2	2	0	0
Ungulata ..	15	11	4	0
Carnivora ..	2	1	1	0
Rodentia ..	1	1	0	0
Chiroptera ..	1	1	0	0
Marsupialia ..	3	1	0	2

COMPARISON OF THE TUBERCULOUS LESIONS IN MAN AND ANIMALS.

While, it may be said, there are no essential differences in the modes of invasion, progression and termination, or in the histological features of tuberculosis in man and animals, the lesions of the developed disease differ considerably in macroscopical characters and in anatomical distribution in the different species of animals. In each species of animals the tuberculous lesions have characteristic peculiarities. These have been described in detail and the chief differences are here summarized.

In man tuberculous lesions are in general liable to caseate and tend to break down to form caseo-purulent abscesses in the lymphatic glands, cavities in the lungs and ulcers in the intestines. The tendency to abscess formation, a characteristic of primate tuberculosis, is very pronounced in monkeys ; the spleen, for example, of a rhesus monkey which has died of general tuberculosis always contains caseo-purulent abscesses, which often entirely replace the splenic substance ; glands also and even whole lobes of the lungs may be converted into abscesses. In the ox, on the other hand, tuberculous lesions are as a rule voluminous, hypertrophic, densely fibroid and calcareous. Caseo-purulent mortary abscesses are not infrequent, but ulceration and cavity formation are less common than in man, owing, no doubt, to the capacity of bovine tissues to surround the lesion with dense walls of fibrous tissue. In bovine lesions calcification appears early and is a prominent feature even in actively progressive lesions. In human tuberculosis calcification and fibrosis are late phenomena and usually indicate arrest or cure of the tuberculous process.

The most striking and characteristic difference, however, between tuberculosis in man and in the ox respectively is the formation in the latter of pediculated or sessile mushroomed growths on the serous surfaces. These, generally described as 'perlsucht' growths, begin, as Cobbett has pointed out, in the villous processes which are situated on the serous surfaces ; these villi are not developed to the same extent in other animals and are not present in man. In tuberculous bovines the villi hypertrophy and little grey tubercles appear in them ; these tubercles enlarge, coalesce, and eventually form characteristic dense outgrowths composed of fibroid tissue infiltrated with calcareous grains. It may be mentioned here that in the spontaneously tuberculous rabbit, fibro-caseous perlsucht-like growths are frequently seen on the diaphragm and mediastinal pleura.

In the fowl the tuberculous lesions, situated usually in the abdominal organs, often resemble neoplasms and are composed of a dense dry yellow friable caseous substance which rarely softens or becomes calcified. In the horse also the tuberculous lesions show a great resemblance to new growths and generally have their seat in the abdomen. During the period before M'Fadyean discovered their nature the tumours were described as lymphadenomata ; they often attain enormous dimensions and are white, homogeneous, dense and poor in bacilli ; occasionally soft caseous foci are found and these are rich in bacilli. In the dog the pleura and pericardium are frequently affected and the lesions have a sarcomatous aspect; in other instances the lungs, which are more frequently affected than other organs, may contain cavities. In carnivorous animals—Canidæ and Felidæ—cavernous pulmonary tuberculosis is comparatively frequent. Also in the goat pulmonary lesions exhibit a strong tendency to break down with the formation of cavities.

Tuberculosis in the guinea-pig is distinguished from tuberculosis in other animals by the diffuse necrotic or caseo-necrotic areas in the liver and spleen, which become greatly enlarged, and the tendency in acute experimental infections to pleural effusion.

Tuberculosis in the pig is generally of alimentary origin, and in severe cases is characterized by a peculiar caseous degeneration of the alimentary, particularly the submaxillary, lymphatic glands, firm fibrocaseous or necrotic nodular growths of varying dimensions in the spleen and tuberculous—often caseous—pneumonia.

The white rat is highly resistant to tubercle bacilli introduced by feeding and subcutaneous inoculation, but can be fatally infected by intraperitoneal inoculation. The bacilli multiply in the tissues, without, however, producing any cellular reactions visible macroscopically. The rat tolerates the toxins of the tubercle bacillus, and death is the result of the multiplication of the bacilli in the cells of vital organs. The white mouse reacts in much the same way as the rat, but is less resistant and may show definite lesions in the organs, particularly in the lungs. In other mammals, it should be added, infection by the tubercle bacillus is not always followed by the production of characteristic tuberculous lesions. Tubercle bacilli may exist in the glands or organs of human beings and animals for long periods without causing any cellular reaction, or at most trivial inflammatory or degenerative changes.

Certain species of animals—guinea-pig, monkey, ape, dog, rat and mouse—and also man react in identical manner to the two mammalian types of tubercle bacilli. Anatomical differences in tuberculous lesions in different species of animals are due, therefore, as Cobbett has stated, not to differences in the bacilli but to differences in anatomical structure of the animals and in the manner of their response to the irritant action of the tubercle bacillus.

References.

Cobbett, L., 1913, *J. Comp. Path.*, **26,** 33; 1926, *ibid.*, **39,** 142; 1917, *The Causes of Tuberculosis*, Cambridge (Univ. Press).

Dammann & Stedefeder, 1909, *Deuts. tierärztl. Wschr.*, **17,** 345.

Eastwood, A. & Griffith, F., 1914, *Rep. Loc. Govt. Bd. Publ. Hlth.*, n.s., No. **91.**

Fox, H., 1923, *Disease in Captive Wild Mammals & Birds*, Philadelphia (J. B. Lippincott Co.); 1928, *J. Amer. Vet. Med. Ass.*, **72,** 792.

Fullerton, A. G. K., 1902, *J. Comp. Path.*, **15,** 102.

Griffith, A. S., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **1,** 386; 1913, *J. Path. Bact.*, **17,** 323; 1917, *ibid.*, **21,** 329; 1924, *Vet. J.*, September; 1925, *J. Comp. Path.*, **38,** 157; 1926, *ibid.*, **39,** 71; 1928^{1}, *ibid.*, **41,** 53 and 109; 1928^{2}, *J. Hyg.*, Camb., **38,** 198.

GRIFFITH, F., 1911, *Royal Commission on Tuberculosis*, Final Rep., Appendix **4**, 4 and 149.
DE JONG, A., 1910, *Ann. Inst. Pasteur*, **24**, 895.
LUCAS, N. S., 1925, *J. Path. Bact.*, **28**, 123.
M'FADYEAN, J., 1900, *J. Comp. Path.*, **13**, 59; 1902, *ibid.*, **15**, 158; 1918, *ibid.*, **31**, 225; 1924, *ibid.*, **37**, 44.
MALM, O., 1912, *Zbl. Bakt.*, Abt. I, Orig., **65**, 42.
MOELLERS, 1912, Rep. *Internat. Tuberc. Congr.*, Rome, 95.
NOCARD, 1894, *Les tuberculoses animales*, Paris; 1896, cited from *J. Comp. Path.*, **9**, 173; 1898, *Ann. Inst. Pasteur*, **12**, 561.
OSTERTAG, 1903, *13th Int. Cong. Hyg.* (*Demogr.*), Brussels, 1903.
RABINOWITSCH, L., 1906, *Deuts. med. Wschr.*, **32**, 866; 1907, *ibid.*, **33**, 1810.
RABINOWITSCH-KEMPNER, L., 1921, *Z. Tuberk.*, **34**, 570.
SCHORNAGEL, 1914, *Tijdschr. v. Veeartsenijk.*, Jan., Feb.
SCOTT, H. H., 1927, *Proc. Zool. Soc.*, Lond., 174; 1928, *ibid.*, 85.
STABLEFORTH, A. W., 1929, *J. Comp. Path.*, **42**, 91.
THIERINGER, H., 1911, *Berl. tierärztl. Wschr.*, **27**, 234.
TITZE, C., 1907, *TuberkArb.*, Berl., **6**, 215.
VALLÉE, H. & PANISSET, L., 1920, *Les tuberculoses animales*, Paris (O. Doin et fils).
WILSON, G. S. & LOVELL, R., 1928, *J. Path. Bact.*, **31**, 583.
ZWICK & ZELLER, 1913, *Arb. GesundhAmt.*, Berl., **43**, 483.

Analysis of Pathogenic Action.

DISTRIBUTION OF TUBERCLE BACILLI.

BY S. R. GLOYNE.

Experimental investigations on the viability of the bacillus outside the body have taken place along three main lines: (1) The prevalence of the bacillus in places where infection by man or animals is likely to have occurred, (2) The length of time the bacillus can remain alive in such places, and (3) Biological researches on viability as part of the life-history of the bacillus. The first two questions are intimately bound up with that of the prevalence of the organism in human and animal secretions and excretions. In most of the earlier research work, investigators were content merely to demonstrate the existence of viable bacilli outside the body without regard to length of time the bacilli had remained alive. In the account which follows only those results will be quoted which are based upon animal inoculation experiments.

Viable tubercle bacilli have been sought for in the following situations:

Sputum and saliva. It is, of course, well known that tubercle bacilli are constantly present in the sputum of phthisical patients. Brown and Petroff (1919) found tubercle bacilli in the saliva of phthisical patients. Gloyne (1922) also found bacilli in the mouths of patients with pulmonary tuberculosis. Crockery used by phthisical patients also retain tubercle bacilli. Brown and Petroff obtained bacilli from the washings of spoons, forks, glasses and cups used by patients, but not from knives and plates. Taylor (1921), Cumming (1920) and Gloyne (1922) also found spoons to be infected. The latter further showed that the cases with large amount of sputum were more liable to infect these utensils than were those with scanty sputum. The act of kissing a sterile Petri dish was found to cause discharge of tubercle bacilli from the mouth (Brown and Petroff). A tooth was also found infected by Brown and Petroff.

Hands. Cumming showed that the hands of phthisical patients were infected, but Brown and Petroff, though confirming this finding when the patient coughed into his hands, were unable to trace any passage of bacilli to a second person when the infected patient shook hands. Door-knobs were found infected by Rogers (1920).

Telephones. Brown and Petroff failed to obtain evidence of infection.

Flies. Brown and Petroff found virulent tubercle bacilli in the bodies of flies fed on tuberculous sputum, but guinea-pigs whose food had been contaminated by these flies crawling over it failed to develop tuberculosis. Gloyne, examining the maggots of blow-flies fed on meat heavily infected with tubercle bacilli, found bacilli adhering to the cuticle of the maggots, but failed to find them in the interior.

Pillow-cases and magazine covers. These were found by Rogers (1920) to be infected.

Dust. Cornet (1904) believed, as the result of numerous experiments, that it was not possible to find viable tubercle bacilli in the streets and in other open places exposed to the adverse conditions of wind, rain, sunlight and diffuse daylight. The organisms are rapidly destroyed—perhaps in a few hours. In dwellings where these conditions do not obtain and massive infection from infected persons is likely, the case is different. Cornet inoculated into guinea-pigs the dust from hospitals, asylums, prisons, dwelling-houses, &c. His results are given in the following résumé :

Number of tests made	147
Number in which at least one animal was tuberculous	40

Positive results have also been obtained by Kirchner (1895), Coates, H. (1901), Wagner (1903) and Rogers (1920), and negative by Brown, Petroff and Pesquera (1920).

It will be seen that in these tests the experimental conditions have been extremely variable. B. Lange (1926) has attempted to eliminate some of these variable factors by means of graded tests as follows. Two sets of experiments were made : (1) with an estimated number of tubercle bacilli suspended in saline, and (2) with sputum in weighed amounts emulsified in saline. Various dilutions of these were then made, spread in thin films and exposed to (*a*) diffuse daylight, and (*b*) darkness for a series of days. In the series of experiments with bacilli suspended in saline, a film containing 1,000 bacilli kept in the dark for 6 days produced tuberculosis in the guinea-pig, whilst none of the dilutions in the daylight test showed viability. In the series of tests with weighed sputum the dilution of 1 in 10 exposed to diffuse daylight for 18 days and the dilutions of 1 in 100 kept in darkness for the same length of time showed living bacilli on inoculation.

RESISTANCE TO DESTRUCTION OF TUBERCLE BACILLI.

Action of light. In direct sunlight, Cornet (1904) quotes Koch and others to the effect that tubercle bacilli ' die within a period varying from a few minutes to several hours ' when exposed in films. He states that direct sunlight killed tubercle bacilli cultures in 2 hours, but when protected from direct rays of the sun they survived up to 5 days. Bacilli in thin films of sputum are said by Calmette (1928) to be destroyed in 10 minutes. Le Noir and Camus (1908) examined the dust from hospital wards by exposing it to April sun and light, through glass, for 5 days, a control portion being kept for the same length of time in the dark. In both portions one test out of five was positive with the guinea-pig inoculation test. Caldwell (1925) exposed sputum mixed with sterile dust to direct sunlight under typical spring weather conditions in Arizona and obtained positive results up to 72 hours.

A good deal of this early work was done with exposure through glass. Howze (1926) tested the effect of ultra-violet light with a mercury quartz lamp, and found that 5 minutes exposure of tubercle bacilli at a distance of 10 inches is sufficient to cause death. Eidinow (1927) found 10 minutes exposure to be the lethal dose, but that 30 to 60 minutes were required if the rays were transmitted through ' vita glass '. Bacilli mixed with blood or serum and exposed in a thin film were not killed.

Action of heat. Summarizing numerous experiments by different workers, Cornet (1904) says a temperature of 55° C. kills bacilli in sputum in 6 hours ; and of 95° C. in milk in 1 minute. The conditions under which the different experiments were made were very variable. No comment is made as to different behaviour of bacilli in a dry and moist environment respectively. Calmette's (1928) figures for a moist environment agree fairly closely with those of Cornet, but are definitely higher for a dry environment.

Action of cold. There seems to be a dearth of investigations on this point. It is stated by Calmette (1928) that even liquid air will not destroy the viability or virulence of tubercle bacilli.

Action of desiccation. Again the results are variable. Most of the work appears to have been done with dried sputum in layers of different thickness. Positive results have been recorded after drying from 2 to 8 months. Variations in temperature, in exposure to light, thickness of film, &c., during the experiments need to be taken into account.

Action of decomposition. Cornet quotes widely differing results from various sources to the effect that the tubercle bacillus is overwhelmed and destroyed by saprophytic and putrefactive organisms in a period ranging from a few days to 10 months, depending on experimental conditions. Special importance might be attached to the effect of these putrefactive organisms on the tubercle bacillus in buried cadavera, but Cornet only quotes two authorities whose results showed living tubercle bacilli after 23 and 167 days respectively. Calmette (1928) records positive results in wet mud after 4 months, and in garden soil after 7 months.

Deprivation of nutriment. Gloyne (1928) attempted to establish ' zero conditions ' by keeping tubercle bacilli in the dark suspended in triple distilled water, sealed in vaccine ampoules packed round with ice. At the end of 12 weeks the bacilli (a virulent human strain) were still virulent for the guinea-pig.

REFERENCES.

BROWN, L. & PETROFF, S. A., 1919, *Contributions to Medical and Biological Research*. New York (Hoeber), **1**, 359.

BROWN, L., PETROFF, S. A. & PESQUERA, G., 1920, *Amer. Rev. Tuberc.*, **3**, 621.

CALDWELL, M. E., 1925, *J. Infect. Dis.*, **37**, 465.

CALMETTE, A., 1928, *L'Infection bacillaire et la tuberculose*, Paris, 3rd ed.

COATES, H., 1901, *Dissemination of Pulmonary Tuberculosis by Means of Infected Dwellings*, Thesis, Victoria University, Manchester. Quoted in *Tubercle*, Lond., 1928, **9**, 561.

CORNET, G., 1904, *Tuberculosis and acute general miliary Tuberculosis*, American Edition by W. B. James and A. Stengel, Philad., N.Y. and Boston.

CUMMING, J. G., 1920, *J. Amer. Med. Ass.*, **74**, 1072.

EIDINOW, A., 1927, *Brit. Med. J.*, ii, 160.

GLOYNE, S. R., 1920, *Tubercle*, Lond., **2**, 12 ; 1922, *ibid.*, **3**, 497 ; 1928, *ibid.*, **9**, 573.

HOWZE, H. H., 1926, *Amer. Rev. Tuberc.*, **13**, 470.

KIRCHNER, M., 1895, *Z. Hyg. InfektKr.*, **19**, 153.

LANGE, B., 1926, *Z. Hyg. InfektKr.*, **106**, 1.

LE NOIR & CAMUS, J., 1908, *C.R. Soc. Biol.*, Paris, **65**, 622.

ROGERS, J. B., 1920, *Amer. J. Publ. Hlth.*, **10**, 345.

TAYLOR, H. L., 1921, *Amer. Rev. Tuberc.*, **5**, 351.

WAGNER, F., 1903, *Zbl. Bakt.*, Abt. I, Ref., **33**, 449.

TOXIC SUBSTANCES OF THE TUBERCLE BACILLUS.

BY A. STANLEY GRIFFITH.

The pathogenic peculiarities of tubercle bacilli are due to toxic substances which they secrete in the course of their growth and multiplication or which are liberated on the death and disintegration of the bacilli.

The toxin of the living tubercle bacillus is a protoplasmic irritant poison and, according to its potency and concentration and the kind of cell involved, either causes coagulation necrosis of the protoplasm and death of the cell or exerts an irritant stimulant action leading to cellular proliferation. Usually there is a combination of cell necrosis and proliferation, the relative amounts of which determine the character of the resulting process, whether mainly caseo-necrotic and destructive or mainly formative and neoplastic.

The earliest visible manifestation of the reaction of the tissues to invasion by the tubercle bacillus is the tubercle, a minute transparent grey granuloma, composed typically of epithelium-like cells, giant cells, and, occurring chiefly around the periphery, lymphocytes and polymorphonuclear leucocytes. The histogenesis of the tubercle has been the subject of much discussion and study. According to the present generally accepted view the first cellular element entering into the formation of the tubercle is the damaged endothelium or epithelium cell. The action of the toxin on these cells causes swelling of the protoplasm, amitotic division of the nuclei—which assume irregular shapes, stain more deeply and allow escape of chromatin—fatty degeneration and a slowly extending coagulation necrosis. Neighbouring cells and underlying connective tissue cells are affected in a similar manner and they merge into one another, losing individuality and forming a protoplasmic mass containing nuclei—the so-called giant cell. Other cells not so intensely acted upon by the toxin are stimulated to proliferate by indirect division (karyokinesis). Thus is formed around the invading bacterium a microscopic mass of ' epithelioid ' cells and cell fusions, which is the primordial tubercle. This tubercle exerts a local irritant action and very soon becomes surrounded by wandering cells—lymphocytes and polymorphonuclear leucocytes—which are attracted to the focus both by the toxins of the bacilli and by the primarily damaged endothelial cells. The amount and character of this leucocytic invasion varies according to the species of animal and the relative virulence of the bacilli or relative resistance of the host. The lymphocytes generally preponderate in the less acute and the polymorphs in the more acute inflammatory conditions. When the lymphocytic invasion is abundant it gives rise to the appearance of a lymphoid follicle (follicular tubercle). When, on the other hand, leucocytes are abundantly present the lesion becomes purulent. Leucocytes and lymphocytes are very sensitive to the action of the toxin and in acute lesions invariably break down.

The subsequent changes in the tubercle depend upon the degree of relative toxicity of the bacillus. When the action of the toxin is very intense there is rapid necrosis of the cells without giant cell formation, abundant multiplication of bacilli and rapid extension of the focus. Generally the process is more deliberate. The tubercle bacilli proliferate, though slowly, and many are killed by phagocytic action ; owing to their fatty waxy envelope tubercle bacilli are not easily digested and their disintegrated remains may be seen in the giant cells. Coagulation necrosis slowly extends to all the primary cells of the tubercle and at this stage there is caseation. Before this some of the bacilli have escaped into the surrounding tissue and given rise to other similar foci. These fuse and by their union produce the miliary tubercle or grey granuloma, which very soon, owing to caseous degeneration, becomes opaque and yellow in the centre. Caseous degeneration is the result mainly of the action of the endotoxins liberated on the disintegration of the tubercle bacilli ; possibly the enzymes of the disintegrated leucocytes contribute to its formation. Caseous substance is a cheesy material composed mainly of coagulated protein and finely divided fat. Caseous matter may soften and rupture externally or into a body cavity, or it may become calcified and completely encapsulated and no longer contain living tubercle bacilli. These are two modes of termination of the tuberculous process. Another mode is by fibrosis. In chronic tuberculosis there is always reaction around the tubercle with inflammatory changes leading to the production of fibrous tissue. New fibrous tissue is produced from plasma cells, endothelial cells and connective tissue cells. If the irritation is of a mild type the focus may be completely transformed into a fibrous nodule. In progressive chronic infection fibrosis around the focus is always incomplete and the new fibrous tissue is continually destroyed.

The toxins of the tubercle bacillus do not readily diffuse, and it is remarkable how extensive local and general tuberculosis may be in animals before signs of ill-health are observed. The death of a tuberculous animal appears to be the result rather of interference with the physiological activities of vital organs by necrosis or by replacement with tuberculomatous masses than of toxæmia. The fever which arises in the course of the disease is probably caused by the products of the metabolism of the bacilli.

While visible lesions—tubercles, nodules and masses—are the usual outcome of an infection with tubercle bacilli they are not invariable. Landouzy and his pupils have described in man a generalized non-follicular bacillosis, a form of tuberculosis without tubercle formation. Many investigators have shown that the lymphatic glands of children and adults who are entirely free from macroscopic lesions may harbour living bacilli, but there is no exact information available as to the length of time these may remain latent in human tissues without producing tissue changes. There is no doubt, however, that in tuberculous, or in highly resistant or partially immunized animals, tubercle bacilli may live and multiply without producing the characteristic lesions. This multiplication without lesions has been observed particularly in animals inoculated with bacilli of low virulence.

Some species of animals, notably the rat and the mouse, exhibit a high degree of tolerance towards the toxins of the tubercle bacillus, and die as the result of the multiplication of tubercle bacilli in the cells of the body without typical tubercle formation. Profuse intracellular growth of tubercle bacilli is a common cause of death in animals after inoculation with avian bacilli but is unusual after mammalian bacilli.

The presence of poisonous substances, or *endotoxins*, in the bodies of tubercle bacilli can be demonstrated by injecting into animals suspensions of dead whole bacilli, dried and ground-up bacilli or bacilli from which the fats and waxes have been extracted. When inoculated subcutaneously into healthy animals dead tubercle bacilli produce a local inflammatory infiltration which quickly becomes a sterile abscess; this ruptures spontaneously and heals completely; the adjacent glands do not enlarge or caseate and the general health of the animal is unaffected.

Inoculated intravenously or intraperitoneally dead tubercle bacilli exert a general irritant action which is manifested by inflammatory and parenchymatous changes in the organs, and cachexia, often terminating in death. Large doses of dead tubercle bacilli inoculated intravenously may cause acute pneumonic consolidation and tubercle formation. Inflammatory and specific changes may also be produced in the lungs by inhalation of dead bacilli. If a coarse suspension is inoculated intravenously and the animal does not die acutely, typical tubercles are formed, which are indistinguishable from the chronic tubercles produced by living bacilli. Dead mammalian bacilli can produce consolidation and typical caseous tubercles in the lungs of fowls, and death from cachexia.

Similar effects (tubercle formation, cachexia and death) may follow the injection of living bacilli into animals which are not susceptible, as for example, bovine and human bacilli into fowls, human bacilli intravenously into calves and rabbits, or avian bacilli into guinea-pigs.

The fundamental characteristics of lesions produced by dead or by living non-virulent bacilli are that they remain localized and are not transmissible in series.

SYMBIOSIS.

BY A. STANLEY GRIFFITH.

Tuberculous lesions not in communication with the exterior rarely contain organisms other than tubercle bacilli. When, however, a necrotic or caseous lesion ulcerates, it invariably becomes secondarily infected with other microbes. These associated bacteria may be saprophytes which grow in the pus and play no part in the pathological process, or they may be pathogenic organisms which invade the tissues and contribute to the destruction caused by the tubercle bacilli. The former association is designated a ‘ secretion-symbiosis ’, the latter a ‘ tissue or infectious symbiosis ’.

VIRULENCE OF TUBERCLE BACILLI.

BY A. STANLEY GRIFFITH.

Experiments in animals have shown that tubercle bacilli derived from warm-blooded vertebrates can be divided according to their pathogenicity for certain species of animals into three well-defined types, namely, the human, bovine and avian types. Different strains of each of these types obtained from their normal habitat, that is to say, bovine strains from bovine tuberculosis, human strains from human pulmonary and glandular tuberculosis, avian strains from avian tuberculosis, are remarkably uniform in virulence. Although the anatomical pathological picture of the disease in each species may show wide variation, these variations are not correlated with differences in virulence of the bacilli found in the lesions.

Natural variations in virulence. Considerable natural variations in virulence do, however, occur. From certain chronic tuberculous affections in man and animals a large number of strains of bacilli have now been obtained which have exhibited less than the usual virulence for experimental animals. This modification of pathogenic properties has been observed frequently in strains from human lupus and equine tuberculosis, and rarely in other chronic conditions, namely, bone and joint, pulmonary, peritoneal and glandular tuberculosis in man and tuberculosis in swine. All three types may show this attenuation in virulence. In man the attenuated strains were either of the human or the bovine type ; in the horse they were of the bovine type and in the pig they were of the bovine or avian types.

The degree to which the bacilli were lowered in virulence has shown considerable variation. Some strains were only slightly less virulent than the standard; others were highly attenuated, and between these were all grades of intermediate virulence. The virulence of some of the modified bovine strains was found not to be fixed and could be increased to standard by a single passage or by several passages through the body of the calf or the rabbit. Other strains retained their lower degree of virulence after the most prolonged residence in the animal body, and in these instances the modification of pathogenic properties appeared to be permanent.

The modification has been described as an attenuation. This conception is correct only in regard to the behaviour of the bacilli towards experimental animals. The bacilli in lupous lesions and in equine tuberculosis had certainly not lost their virulence for their hosts, since in these conditions the disease was progressive.

The cause of the modification of pathogenic properties is not well understood and further observations on this point are necessary. The fact that it occurs in progressive internal lesions as well as in skin tissues seems to rule out the action of physical agents such as light, temperature and the chemical substances used in the treatment of external tuberculosis, and to indicate that it is a vital phenomenon, or adaptation, the result of interaction between the bacilli and the defensive forces of the body.

Experimental variations in virulence. (a) *In the animal body.* Attenuation of virulence similar to that occurring naturally has not been observed under experimental conditions.

(b) *In culture.* When maintained on serum or egg, tubercle bacilli, whether of the human, bovine or avian types, retain their distinctive pathogenic peculiarities for many years. The author found several bovine strains which he had kept under cultivation for from 14 to 17 years still highly virulent for the guinea-pig and the rabbit. Two strains of human type had not apparently lost any virulence for the guinea-pig after 13 and 14 years' cultivation. An avian strain after 6 years' subcultivation was found to be still highly virulent for the fowl. On the other hand, a human strain originally virulent has almost completely lost its virulence for the guinea-pig after 13 years' cultivation on egg. The strain has not regained virulence after prolonged passage through the guinea-pig. Two avian strains have lost their virulence for the fowl after 13 years' subculture.

Other observers have also reported considerable loss of virulence in tubercle bacilli under artificial cultivation. The author has examined three of these strains, namely the Arloing-Courmont strain and two of Raw's strains obtained from the National Collection of Type Cultures (one labelled human, the other bovine), and in addition a strain labelled ‘Bovine Pasteur’. These four strains exhibited the cultural characters and serological reactions of avian tubercle bacilli; they were not virulent for the fowl, but they were still pathogenic for the rabbit, producing joint lesions after intravenous inoculation. These strains must be regarded, therefore, not as examples of attenuated mammalian but of avian tubercle bacilli, attenuated through prolonged subcultivation, 15 years or more. Beck (quoted from Cobbett) thought that glycerin in culture media caused a loss of virulence, but F. and A. S. Griffith and Cobbett, working for the Tuberculosis Commission, were unable to find evidence of it. F. Griffith subcultivated three bovine strains on glycerin media for 3 to 4 years and found them to be equal in virulence to the same strain grown in parallel series on serum alone. A. S. Griffith (1922) tested a bovine strain which had been subcultivated for 15 years on glycerin broth. It was still virulent for rabbits, but was definitely less so than standard bovine viruses. This strain was increased in virulence by passage through rabbits but did not quite attain the full virulence of the bovine type.

It is evident from these results that the tubercle bacillus is a remarkably stable organism. The bovine type of bacillus appears to retain its specific virulence for a greater length of time than the avian or the human type. The avian bacillus, after losing virulence for the fowl, retains some pathogenic power for the rabbit for an indefinite period.

Influence of bile. Calmette and Guérin (1908–14) have stated that when bovine tubercle bacilli are grown upon a medium (potato or agar) saturated with pure bile glycerinated to 5 per cent. they gradually diminish in virulence and eventually lose all power of producing tuberculosis in the highly susceptible monkey or guinea-pig. According to these authors they still preserve their antigenic properties and when suitably administered can produce a very high degree of immunity in animals of the bovine species. This complete attenuation of virulence has been accomplished with, so far as the writer is aware, only one, the well-known B.C.G., strain.

A. S. Griffith has grown bovine, human and avian bacilli on bile egg (7·5 per cent.) or bile potato for periods varying from 1½ to 3½ years. The virulence of a bovine strain kept for 3½ years on bile egg has been considerably reduced, but it is still capable of producing general progressive tuberculosis in the rabbit and guinea-pig and, moreover, has not acquired the cultural characteristics of the B.C.G. strain. An avian strain grown for 3¼ years on bile media is still virulent for the fowl. It is of interest to mention that the B.C.G. strain was practically avirulent for the guinea-pig after only three years' cultivation on bile media.

NATURAL RESISTANCE IN MAN.

BY A. STANLEY GRIFFITH.

No race of mankind is immune against tuberculosis, but different races vary widely in degree of susceptibility. Races previously free from tuberculosis are highly susceptible and infection in them is usually followed by rapid generalization and death from acute tuberculosis; the result is comparable to experimental tuberculosis in the monkey or the guinea-pig. Communities, on the other hand, which have long been exposed to infection and conditions of life favouring the spread of tuberculosis react to infection in a very different manner. Though the opportunities of acquiring the bacillus are abundant and the incidence of infection high (up to 90·0 per cent. in adults in densely populated areas in Europe), the mortality from the disease is comparatively low. Evidence of a high degree of natural resistance is furnished by numerous instances in children and adults in which calcareous and encapsulated tuberculous lesions have been found which no longer contained living tubercle bacilli. This greater resistance of infected communities over virgin races may be due chiefly either to a racial immunity brought about by a process of weeding out of the more susceptible strains of human beings, the resistant individuals surviving, or to an acquired immunity produced by slight natural infections which have been overcome.

In civilized communities susceptibility to tuberculosis is high in infants and young children and diminishes as age advances, but there are wide variations in susceptibility between different individuals of the same race and age.

Naturally acquired resistance. There is little doubt that normal resistance may be raised by an infection which has been successfully localized. It also seems certain from numerous experimental observations that so long as there is an active though minimal lesion in the body the danger of a subsequent infection is lessened.

Causes of break-down of resistance. Many diseases diminish the resistance of the body to tuberculosis. The more important of these are alcoholism, syphilis, the infectious fevers, diabetes, and the deficiency diseases generally. Conditions which diminish resistance to disease in general are considered to predispose to tuberculosis. Pregnancy may delay or arrest the progress of tuberculosis but after parturition the disease usually runs a rapid course. Injuries may light up latent infections or may produce conditions favourable for the settlement and growth of tubercle bacilli, as in traumatic inflammation of bones and joints. Crowded life in close, confined and dusty atmospheres is conducive to the development of tuberculosis, probably mainly because the opportunities of becoming infected are great. The inhalation of dust from hard silicious stones causes damage to the lung and greatly predisposes to a form of pulmonary tuberculosis, which, however, has a low degree of infectivity owing to its slight tendency to ulceration.

REFERENCES.

CALMETTE, A. & GUÉRIN, C., 1908, *C.R. Acad. Sci.*, Paris, **147**, 1456; 1909, *ibid.*, **149**, 191; 1911, *Ann. Inst. Pasteur*, **25**, 625; 1913, *ibid.*, **27**, 162; 1914, *ibid.*, **28**, 329.

GRIFFITH, A. S., 1922, *J. State Med.*, **30**, 139.

Allergy and Immunity in Tuberculosis.

By W. H. TYTLER.

ALLERGY.

The term *allergy*, or *bacterial allergy*, has, in recent usage, been increasingly restricted to the condition responsible for the class of reactions of which the tuberculin reaction is the best known and most widely studied example. This type of hypersensitivity is characteristic for microbic infection, and the reactions to which it gives rise are predominantly inflammatory in nature. It appears to be distinct, at least superficially, from anaphylaxis, and from the hypersensitivity of asthma and hay fever. Such use of the term *allergy* is a much narrower one than that employed originally by v. Pirquet, and still employed, to some extent, in general discussion of hypersensitivity. But it is a common fate of such terms that their sense is restricted as analysis of phenomena proceeds, and it is highly desirable that there should be a distinctive name for the condition in question, which appears, at present, to be an entity. It is in this limited sense that the term is used in the following discussion.

General Characteristics of the Allergic Reactions.

The predominant phenomena of the allergic condition in tuberculosis, as it is known to-day, were demonstrated in the observations described by Robert Koch (1890, 1891[1]) in his original communications. When tubercle bacilli, living or dead, were injected subcutaneously in the tuberculous guinea-pig, the site of injection showed, within 24 to 48 hours, an intense inflammatory reaction, with hæmorrhage and necrosis ; while the same treatment, in the normal animal, produced, at this stage, no significant reaction. Following these results, injections were made in the human subject, using, at first, extracts of tubercle bacilli, which were soon replaced, however, by the fluid culture media on which the bacilli had been grown. In tuberculous patients there followed an acute rise of temperature, with general toxic symptoms, and an intense inflammatory reaction at the site of lesions, well observed in cases of *lupus*, while there was swelling and tenderness at the point of injection.

The reactions described by Koch represent the three types of allergic reaction to *tuberculin** which are by custom classed as general, focal, and local.

The general reaction.

The febrile and toxic reaction depends on the entry of *tuberculin* into the general circulation, and is obtained by any method of application which leads to rapid absorption—as subcutaneous, intraperitoneal, or intravenous injection, or application to mucous surfaces.

In man, the most prominent symptom is a sharp rise of temperature, usually within 3 to 5 hours, after subcutaneous injection, rising to a maximum at 10 to 15 hours, and falling rapidly to reach normal before the twenty-fourth to thirtieth hour. General toxic symptoms, malaise, headache, nausea, &c., may be marked. In the larger domestic animals the febrile reaction is of the same character, but in small laboratory animals, such as guinea-pigs or rabbits, it is, in general, less regular in type

*The term ' *tuberculin* ', in italics, is used, in this discussion, to indicate the constituent of the tubercle bacillus which elicits the allergic reaction. By ' tuberculin ', in Roman type, is meant any active preparation made from the media of tubercle bacillus cultures. For preparations after definite formulæ, as Old Tuberculin, the respective names, or initials, e.g. O.T., are used.

The focal reaction.

The focal reaction is produced by the same methods which elicit the general reaction, and the two processes appear to be intimately related. Koch's description of the reaction occurring at the site of *lupus* lesions, after subcutaneous injection of tuberculin elsewhere, gives a picture of the most severe grade. In a few hours after the injection the lesion, and the surrounding undiseased tissue, showed congestion and œdema, becoming rapidly more intense, with ecchymosis and vascular stasis, leading to necrosis and sloughing, with final scar formation. As a result, much of the *lupus* tissue was eliminated, and on this Koch based his view that tuberculin was a specific destructive agent for tuberculous tissue. With the smaller modern dosage the reaction, as seen in man, is usually limited to a degree of inflammation causing swelling, tenderness, exudation, increased symptoms, &c., according to the site of the lesion. The reaction occurs characteristically in the more vascular tissue about the lesion.

The focal reaction has not been extensively studied either experimentally or anatomically, but the pathological process seems to be essentially the same as in the case of the local reaction, described below.

The local reactions.

In his first work with human patients, Koch described the occurrence, in reacting cases, of some diffuse inflammatory swelling at the site of subcutaneous tuberculin injection, that is, at the contact between *tuberculin*, and the undiseased tissues of the tuberculous subject. This was soon recognized as a characteristic feature—the ' depot reaction '. Epstein (1891) also noted cutaneous erythema and infiltration about the needle puncture, to which Escherich (1892) gave the name ' stichreaktion ', and this, the first of the cutaneous reactions, was gradually recognized as having considerable diagnostic value.

The v. Pirquet reaction. v. Pirquet (1907[1 & 2]), following the classic study of vaccinia which led to his conception of ' allergy ', applied tuberculin to the skin surface after scarification, as for vaccination, and, in tuberculous subjects, obtained a characteristic reaction. Within 24 to 48 hours there developed a firm, indurated papule, surrounded by an erythematous areola, the inflammatory process reaching its maximum at the second to the fifth day, and disappearing slowly, so that it was still visible after several days, or even weeks. The use of this simple diagnostic test rapidly became world-wide, and has yielded information of inestimable value to the epidemiological study of tuberculosis, while it furnished the chief impetus to the experimental analysis of *tuberculin* sensitivity which has continued progressively since that time.

The intracutaneous reaction. The injection of tuberculin into the skin itself, already used experimentally by Klingmuller (1903), was introduced by Mendel (1908) and by Mantoux (1908) for diagnostic use in man. Old Tuberculin of low concentration is used—usually starting at 1 in 5,000—and about 0·1 c.cm. is injected. The ensuing process is essentially similar to the v. Pirquet reaction. The method permits much more accurate dosage, but even to-day is only slowly replacing the simpler scarification method in clinical work. In cattle, where the v. Pirquet method gives irregular results, the value of the intracutaneous method, employed by Moussu and Mantoux (1908) and by Römer (1909[1]) was soon recognized, and it has gradually supplanted, to some extent, the traditional subcutaneous method.

Römer and Joseph (1909) employed the method extensively in guinea-pigs, in which it gives characteristic and reliable results. Since many of the experimental data recorded are based on its use in this animal, the reaction may be described in detail.

White guinea-pigs are used for preference, and the skin is first depilated, e.g. by freshly prepared calcium hydrogen sulphide. The injection, usually 0·1 c.cm. or less, is made with a fine, short needle, into the superficial layers of the skin, so that a well-defined bleb is formed. With higher concentrations of Old Tuberculin, 1 in 5 to 1 in 50, the tuberculous animal shows, at 24 hours, a raised plaque, 15 to 50 mm. in diameter, the centre of which, 10 to 20 mm. diameter, is hæmorrhagic, and violet-red in colour. This central area is usually sharply demarcated from the marginal zone, which shows firm, œdematous infiltration, usually so intense that the surface is blanched. Outside the plaque there is a third zone of erythema, and, from the three colour zones, the name ' cocarde ' reaction was given by Moussu and Mantoux. At 48 hours, the surrounding erythema is fading, the plaque is usually slightly smaller, but firmer and more sharply defined, while the central area has become frankly necrotic, and, in the course of the next few days, a superficial ulcer is formed, which heals rapidly.

With lower tuberculin concentrations, the central ecchymosis is smaller, and irregular, and may disappear without definite necrosis, while at dilutions weaker than 1 in 200, to 1 in 500, it is seldom seen at all, the reaction showing merely a firm œdematous plaque, often with central blanching. The size of the plaque decreases progressively with descending doses. The reactions are commonly palpable for at least 4 or 5 days, and, where necrosis has occurred, for 2 weeks or more. In the normal guinea-pig, even a 1 in 5 dilution seldom produces a nodule larger than 5 mm., and this has almost disappeared at 48 hours.

The method may be used quantitatively, either for estimation of sensitivity in the animal or for standardization of tuberculin. In the former case the lowest concentration of standard tuberculin giving a definite reaction is determined ; in the latter, the unknown solution is compared, in serial dilution, against similar dilutions of the standard, in the same animal. Measurements of the reaction plaques are best made at 48 hours, when the borders are well defined.

A number of other methods for eliciting the local cutaneous reaction have been devised, of which the best known is the *percutaneous* method of Moro (1908), in which the tuberculin is applied in ointment form. The positive reaction shows diffuse erythema, and an eruption of fine papules or vesicles.

The conjunctival reaction. In this method (Wolff-Eisner, 1907, 1908; Calmette, 1907) 1 per cent. Old Tuberculin is dropped into the conjunctival sac, the surfaces of which, in reacting cases, develop diffuse congestion and œdema, commencing at 5 to 6 hours, reaching a maximum at 6 to 10 hours, and fading after 18 to 30 hours.

The spermatocyte reaction. Long (1924, 1925) employed intratesticular injection in the guinea-pig as a highly sensitive method for experimental work. The positive reaction shows, in addition to acute, diffuse inflammation, a characteristic necrosis of the tubular epithelium, and particularly of the spermatocytes. The animal is killed a month later and the testicle examined histologically, the epithelial destruction being then most obvious.

Local reactions have also been obtained by application of tuberculin to mucous surfaces—nasal, urethral, rectal, vaginal. These methods have been used chiefly in veterinary work, and do not seem to have given constant or reliable results.

Tuberculin Death.

Koch (1891^2) found that the febrile and focal reactions following the subcutaneous injection of small amounts of tuberculin in the tuberculous guinea-pig were not sufficiently reliable to serve as a measure of tuberculin activity, but that with larger doses death was regularly produced in 6 to 30 hours, with a characteristic post-mortem appearance. Further, in animals with well-developed disease, the minimum lethal dose was relatively constant, and the method has formed the basis of tuberculin standardization up to recent years, though now, to some extent, replaced by the intracutaneous method. Koch found that guinea-pigs infected 4 to 5 weeks previously were usually killed by 0·2 to 0·3 gm. of his Old Tuberculin, and always by 0·5 gm., while with more advanced disease death might follow 0·01 gm. It is generally demanded of Old Tuberculin to-day that it should kill within 24 hours at a dose of 0·1 c.cm.

Post mortem, there is found intense congestion at the site of tuberculin injection, hæmorrhagic exudate in serous cavities and diffuse congestion of serous surfaces. The peritoneum shows, particularly over the liver surface, numerous fine ecchymotic flecks, which actually represent fine rings of hæmorrhage and congestion surrounding individual tuberculous lesions. The spleen, in advanced disease is of a diffuse reddish-black colour. The picture is essentially that of intense inflammatory and hæmorrhagic reaction at the sites of all tuberculous lesions. Death is commonly ascribed to toxic absorption from the focal reactions, and these seem sufficient to account for it on the basis of secondary and non-specific effects alone.

Tuberculin death in other animals is usually an accidental and undesired event in the course of experimental work, and has not been greatly studied, but the pathological process seems to be the same as in the guinea-pig.

The reactions to intact bacilli.

Koch's phenomenon, obtained on subcutaneous injection of tubercle bacilli in the tuberculous guinea-pig, seems, in as far as concerns the acute inflammatory phase, to represent essentially the same process as the local reaction to tuberculin, although some still consider it as a separate entity. By certain modes of application, the reactions to intact bacilli differ from those to *tuberculin* in solution, but the differences may be explained on the basis of mechanical factors.

Tuberculin is rapidly absorbed from vascularized tissues, and is rapidly eliminated from the blood by excretion. Hence, subcutaneous injection of the solution gives a rapid general reaction, of relatively short duration, but a weak reaction at the site of injection. Tubercle bacilli, injected subcutaneously, are held at the site of injection, and an intense local reaction is produced. Intraperitoneally, they elicit an equally violent reaction, with purulent and hæmorrhagic exudate (Bail, 1904; Manwaring and Bronfenbrenner, 1913; Rist, Léon-Kindberg and Rolland, 1914^1). Intravenous injection of bacillary emulsions produces local reactions in the lungs (S. Arloing, 1903), or kidneys (Rist and Léon-Kindberg, 1914), but, as the reacting material cannot be rapidly removed by excretion, it may also produce a more marked general reaction than tuberculin by the same route, as Römer (1909^2) found in cattle.

By the intracutaneous method, in which the active material, in whatever form, is only slowly removed from the site of injection, the reaction to bacilli and to tuberculin are the same, both giving the characteristic necrotic lesion. The writer has recently injected each of a series of guinea-pigs with Old Tuberculin, an emulsion of living bacilli, and a purified bacillary protein, and found the reactions indistinguishable macroscopically, or, save for the presence of bacilli, microscopically.

Pathology of the reactions.

Histological study has been largely confined to the lesions of the local skin reactions, and, in general, to the early stages in animals, and the later stages in man. The initial phase is essentially an acute inflammation, with marked vascular dilatation and œdema, and exudation of polymorphonuclear leucocytes and red blood-cells. The subcutaneous or intracutaneous reactions described by Bezançon and de Serbonnes (1914), Rist and Rolland (1914), and Rolland (1914) after injection of bacilli, and by Stewart and Rhoads (1926) with tuberculin, and those examined by the writer with both materials, all showed this process, while the more intense reactions constantly showed superficial necrosis, followed by sloughing and rapid healing.

Intraperitoneal injection of tubercle bacilli in the tuberculous guinea-pig constantly produces a rapidly accumulating purulent exudate. In Long's spermatocyte reaction the testicle shows the same purulent process, but with the added factor of a diffuse necrosis of tubular epithelium, and in the pulmonary reaction to intratracheal injection of bacilli, Bezançon and de Serbonnes (1914) observed a somewhat similar necrosis of alveolar epithelium. There is, therefore, some suggestion of a direct toxic action, and it is to be noted that, in the intracutaneous reaction, the central part of the necrotic block is at first almost devoid of leucocytes, while it has been observed, in all types of the reaction, that the exuded leucocytes undergo rapid disintegration.

In man, cutaneous reaction sites have been examined from about the sixth day, and even at this stage leucocytes are still in evidence (Zieler, 1910[1]). Later, the lymphocyte predominates, and after about two weeks there appear mononuclear cells of the 'epithelioid' type, together with giant-cells, while there may be a focal arrangement of these cell types, suggesting the tubercle of active disease. These lesions, first described by Klingmuller (1902, 1903), have given rise to much controversy, and to complicated theories based on their supposedly specific nature. They were first ascribed to the presence of bacillary particles in the tuberculin, but Zieler (1910[2]) produced them, not only with filtered tuberculin, but with dialysates of tuberculin. A probable explanation is that suggested by Kyrle (1920), that they represent a reaction about areas of necrotic tissue, and Stewart and Rhoads (1926), consider them particularly related to necrotic fat tissue.

Toxicity of tuberculin for the normal animal.

The effects, in the normal animal, of various constituents of the tubercle bacillus have been studied, and some of the fractions appear to have had a certain degree of toxicity. In general, however, large doses have been required, and the effects have not been sufficiently constant or characteristic to suggest that the bacillus produces any substance of the nature of the known bacterial toxins.

It might be expected that Old Tuberculin, with its high concentration of media constituents, would have a considerable effect on normal tissues, but it seems to be well tolerated by the normal subject in relatively large amounts. Sons and Mikulicz-Radecki (1921) quote Bauer as having given 50 gm. to infants, and Ruppel 20 gm. to guinea-pigs, without ill-effect. Schittenhelm and Weichardt (1912) gave 28 c.cm. to a dog without reaction.

In particular, there is no evidence that tuberculin or bacillary constituents, in any amount, can produce, in the normal, a process identical with the allergic reaction of the infected animal. The qualitative difference is better seen when non-specific factors are diminished by the use of purified bacillary fractions. Sabin and Doan (1927) injected rabbits intravenously with large amounts of a purified bacillary protein prepared by Johnson, and found that the cellular response involved chiefly the mononuclear cells of the clasmatocyte type. The writer, in a series of normal guinea-pigs, injected intraperitoneally with a similar protein fraction, found the cellular exudate at 24 hours to consist, in at least 90 per cent., of cells of the macrophage type. The reaction of the tuberculous animal to tuberculin and to these protein fractions shows, on the other hand, a great preponderance of polymorphonuclear leucocytes.

The extent of the difference in reacting power is still better demonstrated by the quantitative relations. Römer and Joseph (1910[2]) estimated the amount of tuberculin required to produce an appreciable intracutaneous reaction in the normal guinea-pig at 10,000 times as great as in the tuberculous animal, while in man the relation was 100,000 to 1. These ratios are somewhat higher than those given by others, but the difference, in any case, is sufficient to show that *tuberculin* has no action in the normal which is any way comparable with its effect in the tuberculous animal.

Reactions in various species.

The allergic reaction to tuberculin has been described in most of the domestic animals—horse, ass, goat, sheep, ox, swine, dog, rabbit and guinea-pig—as also in apes and monkeys. In most, if not all of these animals, the febrile reaction may be obtained, but the local skin reaction varies greatly, and skin sensitivity is commonly less than in man. The guinea-pig is relatively insensitive to the v. Pirquet method, but more sensitive than most other animals to intracutaneous injection. The necrotic reaction is particularly characteristic in the guinea-pig, but may be obtained, with sufficient dosage, in other species. The rabbit has been much used in the study of the febrile reaction, but gives an indefinite skin reaction, with little infiltration. According to Calmette (1928[1]), well marked skin reactions are obtained in the apes, but the lower monkeys react very irregularly. In the fowl, local reactions to avian bacillus tuberculin are obtained by injection in the wattle or comb.

The Nature of the Active Principle in Tuberculin.

The reactions which Koch obtained on the injection of tubercle bacilli in the tuberculous animal led him to the investigation of the reacting substance, and this was first obtained in soluble form by extraction of the bacilli with 50 per cent. glycerin solution. From the necessity for larger yields, this extract was soon replaced by the preparation known as Old Tuberculin. Cultures on glycerin-broth media, after 6 to 8 weeks' growth, were steamed down, with the bacillary mass still present, to one-tenth of the original volume, and the bacilli then removed by filtration. Koch believed that the active substance was extracted chiefly during the concentration process. This preparation has remained, to the present day, the standard for diagnostic use.

Since that time, active solutions have been prepared by many different methods, and may be divided into two general classes :

(1) Those prepared from culture media on which the tubercle bacillus has been grown, and known in general as ' tuberculins ', or ' culture filtrates '. A very large number of such preparations have been described—mostly designed for therapeutic use.

(2) Those prepared by extraction of the bacilli after removal of the media, and here referred to as ' bacillary extracts '. They have been used chiefly in experimental work.

Koch himself (1891[2]) attempted to isolate and to characterize the active principle present in tuberculin, and the information he obtained actually covers, in principle, most of the present-day knowledge on the subject. He found that an active fraction could be separated by a variety of reagents, mostly protein precipitants, and selected alcohol as giving the cleanest and most active product. The purified alcohol precipitate was fifty times as active as the original tuberculin, and gave the ordinary protein reactions. It was partially coagulated by heat when in high concentration, but not in dilute solution, while in 50 per cent. glycerin its activity was unaffected by heating to 160°C. Koch believed the substance to be of protein nature, and probably a type of protein characteristic for bacteria in general. Subsequent chemical investigations of the tuberculins of this type have added, until very recently, little reliable information, while the conflicting results obtained, and superficial assumptions, based on the methods of preparation, have led to much confusion on the theoretical side.

A few years after the first preparation of tuberculin, Koch (1897) produced a new type of active solution, by the aqueous extraction of tubercle bacilli which had been exhaustively ground in the dry state, the preparation being known as 'T.O.'. Since that time aqueous extracts, prepared by various methods, have been widely used as preferable to Old Tuberculin in experimental work, and, to some extent, for therapeutic use. Most commonly, the extracts have been made with weakly alkaline solutions, which more readily remove the protein constituent.

From the chemical standpoint, a characteristic constituent of the bacillus had already been noted by Weyl (1891)—a substance extracted by alkalis and precipitated by acids in the cold. Ruppel (1898, 1900) studied this substance more extensively, found that it was an important constituent of extracts made by Koch's method, and classified it as a 'nucleoprotein'. This seems to have been largely an assumption from its physical characteristics, from its high phosphorus content—about 10 per cent.—and from the presence of free nucleic acid in the extracts, but it has been widely accepted up to the present day, and similar 'nucleoproteins' have been separated from various other bacteria (see Lustig, 1913). Recent work makes it doubtful whether the characteristic water-soluble, acid-precipitable protein contains any important amount of true nucleoprotein.

Various workers have found that the precipitate produced by acetic acid in bacillary extracts (Toenniessen, 1922, 1924; Armand-Delille, 1923; Petroff, 1923; Mueller, 1926), or in tuberculin (Dienes and Schoenheit, 1925; Long and Seibert, 1926[1]), contains the bulk of the material which can elicit the specific skin reaction. In recent work it has been found that this material can be removed entirely by sodium sulphate (Dienes, Schoenheit and Scheff, 1925), or by ammonium sulphate (Seibert and Long, 1926[2]; Henley, Dorset and Moskey, 1927). Seibert and Long found removal complete at three-quarters saturation, while the others have used full saturation.

Johnson and Coghill (1926) have prepared, from bacillary extracts, large amounts of a highly active protein, and stress the importance of avoiding injury by heat or desiccation. They appear to have used a variety of methods, but from the most recent publication (Johnson and Renfrew, 1928[2]), their product ' 304 ', which has been studied by several other workers, consists of the whole acetic acid precipitate from aqueous extracts of ground defatted bacilli. The fat extraction is carried out, for preference, with cold, dry ether only.

Of recent work, the most important is that of Long and Seibert, who worked with large volumes of filtrates from cultures grown on protein-free, synthetic media, the active fraction being precipitated by ammonium sulphate, dialysed, and dried either directly, or with alcohol and ether.

The writer has made a large number of preparations from bacillary extracts by methods comparable to those of other recent workers. The bacilli are washed, dried, roughly defatted with acetone, and thoroughly ground. The dust is extracted with water at 37° C. for 2 to 6 weeks. Extraction at 100° C. either of the original material or of the residue from the 37° extraction constantly removes an important amount of fully active protein, but this product tends to lose its solubility in the dry state, suggesting heat injury. The extracts are centrifuged and filtered until water-clear, and the filtrates precipitated with acetic acid. The precipitate flocculates sharply at pH 3·8 to 4·0, and is redissolved in small volume at pH 5·5 to 6·0, reprecipitated by ammonium sulphate, dialyzed and dried under high vacuum from the frozen state. The yields vary considerably, the 37° extract yielding from 2 to 6 per cent.—usually about 4 per cent.—of the dry weight of the bacilli, while subsequent hot extraction removes another 1 to 2 per cent.

The characteristics of the various active products described may be discussed in detail.

Chemical structure. Koch's alcohol precipitate, bacillary extracts in general, and the acid and sulphate precipitates described by the various workers noted, have given biuret and Millon's reactions. The nitrogen content has usually been from 10 to 13 per cent., while Johnson and Coghill (1926) have purified their product until it contains over 14 per cent.

Seibert (1928[1]) found the water-soluble product from culture filtrates to contain 80 per cent. of protein nitrogen, precipitated by trichloracetic acid, while another 8 per cent., precipitated with the protein by phosphotungstic acid, represented proteose. The writer's products, including those extracted at 100° C. have shown, by the same method, over 90 per cent. protein nitrogen.

Seibert (1928[2]) has recently obtained protein crystals, by the Hopkins method, which show high tuberculin activity.

Heat coagulation. Most writers have described their active preparations as consisting partly or largely of coagulable protein, but it is not always clear whether coagulation was obtained at neutral or at acid reaction. Long and Seibert found a large part of their product coagulable, and Seibert (1928[1]) finds the coagulable fractions to contain 81 to 95 per cent. protein nitrogen, the fraction incoagulable at 120° C. showing 61 per cent. proteose and only 29 per cent. protein nitrogen. In preparations from culture filtrates the writer has obtained similar coagulation, but the products from 37° extracts have never shown any measurable coagulable nitrogen, when heated in a buffered solution at pH 6·5 to 6·8. Coagulation is first obtained at about pH 5·5, the point at which precipitation commences in the cold. Johnson and Renfrew (1928[1]) found that in cultures on synthetic fluid media heat-coagulable protein regularly appeared in the media at the fourth to fifth week of growth.

Dialysis. Koch (1891[1]), Zieler (1910[2]), Löwenstein and Pick (1911) and Zinsser (1921), found the active principle to be wholly or partly dialysable. Recent workers have usually found the reverse, and Seibert and Long (1926[1]) obtained little or no skin reaction with dialysates from the entire culture filtrates, though dialysates from the sulphate precipitates constantly showed some activity. The writer has tested a number of extracts, purified fractions, culture filtrates, and commercial tuberculins, by pressure filtration through collodion sacs. The filtrates have usually been completely inactive for the skin reaction, and any sacs which showed permeability were permeable in about the same degree to crystallized egg-albumin.

All experience of dialysis would indicate that the discrepant results might be explained by differences in the membranes employed, but another factor is suggested by the results of Henley, Dorset and Moskey (1927). With tuberculins prepared from young cultures, the substances eliciting both the skin reaction and the lethal reaction were removed by ammonium sulphate and were non-dialysable (fish-bladders). But in old cultures, which had become acid, the two effects could be separated, the substance giving the skin reaction being held back by the membrane, and precipitated by ammonium sulphate, while the dialysates and the sulphate filtrates contained the lethal principle.

Adsorption. Long and Seibert (1925) obtained no evidence of adsorption of active material by charcoal, but Dorset, Henley and Moskey (1926) found that adsorbents carrying a negative charge—kieselguhr, charcoal, fuller's earth—largely or completely abolished the activity of tuberculin, while calcium carbonate, carrying a positive charge, had no effect. Boquet, Nègre and Valtis (1928) have confirmed this result for charcoal, and in the writer's laboratory it has been found that aluminium hydroxide strongly adsorbs the active protein prepared from bacillary extracts.

Activity of products. No strict comparison of activity, as between the different products described, is possible without a common standard of reference, and most data given are indefinite. Dienes and Schoenheit (1925) say that 0·001 mgm. of their preparation was the minimum for a

' markedly positive reaction '. Long and Seibert's water-soluble fraction gave a skin reaction at a dose of 0·01 mgm., while Seibert's crystallized product was two and a half times as active. The writer's more recent preparations have had an almost constant value such that 5 to 6 mgm. is equivalent to 1·0 c.cm. of Old Tuberculin equal to the Frankfort standard. In fully sensitive guinea-pigs, definite intracutaneous reactions are given at a dose of 0·0001 mgm., while definitely ecchymotic reactions may be given by 0·001 mgm.

Relation of the protein to activity. It has long been recognized that tuberculin activity is destroyed by proteolytic enzymes. Seibert (1926) found this to hold for ferments which destroyed the protein, and Long (1926) found the same for acid-hydrolysis, but Seibert, by treatment of the active fraction with trypsin in neutral solution obtained destruction of the proteose only, and the activity was unaffected. This is strong evidence for the relation of the protein to activity, but it is in some conflict with Seibert's recent results (1928[1]), in which she found that the incoagulable fraction, containing only 30 per cent. protein nitrogen, was about as active, for the skin reaction, as the coagulable portion. The former, however, was definitely more toxic as tested by the lethal reaction, which suggests an analogy with the results of Henley, Dorset and Moskey. The writer's results also support the relationship between the protein and activity. The final preparations, containing 90 to 95 per cent. protein nitrogen, while they show considerable variation in yield, as between different extracts, are remarkably constant in activity, on a dry weight basis. This means that if the active substance is not the protein itself, it must come out, by the methods of separation, in a constant proportion to it, a most unlikely event.

Discussion. There appears to be little doubt that the various active preparations described, from whatever source, are largly of protein nature, though showing a difference from the well-known animal proteins. The writer's products give at least no frank coagulation with heat, though there is evidence of the injurious effects of denaturing influences, not only of heat, but of exposure to concentrated sulphate, as found also by Long and Seibert. The active fraction, however, seems to be more stable than is suggested by Johnson and Coghill.

Whether the fractions separated from culture-filtrates, and those from extracts, are the same, cannot yet be decided, but they seem to be very similar. The optimum precipitation reaction, and the isoelectric point was found by Gabbe (1923) to be the same for both—pH 4·0. Long and Seibert (1926[1]) found the same for their product, and the writer's extracts flocculate regularly at this point.

While the protein molecule appears to carry the active principle, especially for the skin reaction, the results of Henley, Dorset and Moskey (1927) and of Seibert (1928[1]) definitely suggest that activity may also be carried by a lower order of molecule, and may be brought into evidence by the lethal reaction.

Allergy in Tuberculous Infection.

Development of sensitivity.

The interval which elapses between primary infection by the tubercle bacillus, and the development of sensitivity to the degree at which the allergic reaction may be demonstrated, shows, in experimental infection, considerable variation. For natural infection, data are scanty, but here also there appears to be no fixed latent period. The factors which influence the duration of this interval are, in outline, relatively simple—the size of the infecting dose, the virulence of the bacilli, the path of infection, and the natural resistance of the host—while the value given to it will vary with the method used to elicit the reaction.

Information concerning the appearance of the general febrile reaction is not extensive. Boquet and Nègre (1926), in guinea-pigs tested by intraperitoneal injection of 0·05 c.cm. of O.T., found the latent period shorter after large infecting doses than after small, and shorter after a given dose of virulent bacilli than after the same dose of an avirulent strain (B.C.G.). Reactions were obtained at 5 days after 1·0 mgm. of virulent bacilli, and at 12 to 14 days after 0·0001 mgm. With B.C.G., the interval was 5 days after 40 mgm. and 14 days after 0·1 mgm. After 0·01 mgm. virulent bacilli intracutaneously, it was 18 days, after the same dose by conjunctival instillation, 17 to 23 days, and after ingestion of 0·001 mgm., 23 days. In rabbits, the relations were similar, but the latent period was longer throughout the scale. According to Courmont (1927), a febrile reaction may be obtained in the guinea-pig on the third day, if the test dose of tuberculin is large.

From the fact that the febrile reaction appeared suddenly, and in high degree, Boquet and Nègre conclude that tuberculin sensitivity does not intimately depend on the evolution of tissue lesions, and they consider Koch's phenomenon, which appears later than the febrile reaction, as dependent on a separate process. Apart, however, from the unreliability of temperature records in small animals, the febrile reaction is unsuited to quantitative estimation of sensitivity, and results obtained with the local cutaneous reaction must be considered as more significant. It is true that here, also, a rapid increase in sensitivity is seen at the time when the reaction first appears, but the most reliable results indicate that subsequent increase, up to the maximum degree, is a progressive process, and influenced by the progress of the disease. The necrotic reaction, whether to intact bacilli subcutaneously, or to tuberculin intracutaneously, appears to represent only a high degree of sensitivity, and to be dependent on mechanical factors, as already discussed.

Hamburger and Toyofuku (1910), found that after subcutaneous infection of guinea-pigs with 0·5 mgm. of bacilli, an intracutaneous reaction to O.T., 1 in 5, was obtained on the fifth or sixth day, and some ecchymotic reactions were seen on the eighth day. Indefinite reactions occurred on the third day, and they believed that sensitivity developed progressively. Most workers, commonly using smaller infecting doses, describe the intracutaneous reaction as appearing about the tenth day.

Römer and Joseph (1910²) examined a large series of guinea-pigs after subcutaneous infection with doses ranging from 0·1 mgm. down to those of the order of 1 or 2 bacilli, and tested them by repeated intracutaneous injections of O.T. in graded doses—20, 2·0, 0·2 and 0·02 mgm.—a reaction to the smallest dose representing about the maximum sensitivity for this animal. A reaction to 20 mgm. was given at 2 weeks after infections down to 0·0001 mgm., and at 3 to 12 weeks after smaller doses. A reaction to 0·02 mgm., however, was reached only at 9 weeks after 0·1 mgm., and at 15 weeks after 0·00001 mgm. With smaller infecting doses this grade was not reached at all, and with the smallest, producing a 'chronic, latent infection', reactions were not obtained below 2·0 mgm. O.T.

Krause (1916), in guinea-pigs infected with an undetermined, but apparently large dose of virulent bacilli, obtained intracutaneous reactions at 11 days, which increased in intensity up to 30 days, the increase being particularly evident in regard to the extent of necrosis, which was only slight at 18 days. Animals inoculated with an avirulent strain (R 1) showed no reactions at 11 days, non-necrotic reactions at 18 days, beginning necrosis at 25 days, and never gave the same extent of necrosis as seen in the first series.

Parish and Okell (1929) have recently examined the development of sensitivity by methods similar to those of Römer and Joseph. Guinea-pigs infected with 0·5 mgm. of virulent bacilli reacted after 1 week to O.T., 1 in 50, intracutaneously. The subsequent increase in sensitivity was very irregular, the end-point at 2 weeks being from 1 in 50 up to 1 in 4,000, and, at 3 weeks, from 1 in 100 to 1 in 8,000. Their results indicated the most sensitive period as the third week, but it is to be noted that their infecting dose was large.

Bezançon and de Serbonnes (1914) have found that Koch's phenomenon, with subcutaneous reinoculation, is first obtained at the sixteenth to eighteenth day. Debré and Bonnet (1922) injected guinea-pigs intracutaneously with 0·1 mgm. living bacilli, and repeated the dose at 10-day intervals. The second and third doses elicited evidence of allergy, but no necrosis. The fourth (30 days) sometimes, and the fifth (40 days) constantly gave a typical Koch's phenomenon. They found the cutaneous reactions to living bacilli and to tuberculin to be closely parallel.

While the bulk of the experimental evidence is derived either from the febrile reaction, or from the local cutaneous reaction, there is little doubt that, in the fully developed allergic condition, the tissues of the entire body become sensitive, though it does not appear that they do so at a uniform rate, or to uniform degree. The focal reaction suggests, indeed, that those tissues in close relation to sites of infection, and thus exposed to a greater concentration of bacillary constituents, become more sensitive than tissues elsewhere, although an alternative explanation is discussed later. Stewart (1925) has shown that the tissues at the site of infection become allergic more rapidly than those at a distance. Guinea-pigs were infected by injection into one testis, and subsequently tested by injection of tuberculin into both testes, and intracutaneously. Evidence of reaction was obtained as early as the third day in the infected testis, and not until the sixth day in the other, while the intracutaneous reaction first appeared at the eleventh day.

In man, where only natural infection can be studied, data concerning the development of sensitivity are difficult to obtain. Debré, Paraf and Dautrebande (1921), however, estimated the incubation period in infants of tuberculous mothers, kept in the Laennec Hospital under conditions which practically excluded infection subsequent to their entry. In many infants the v. Pirquet reaction was negative on admission, but became positive after from 1 to 4 months.

In the natural course of primary infection the development of allergy may be clearly indicated by clinical symptoms. In the guinea-pig there is a well-marked phase, usually commencing during the third week after infection, in which the animal loses weight and vitality, while the local lesions increase rapidly in size. This is the period at which tuberculin sensitivity first develops in high degree, and there can be little doubt that spontaneous allergic reactions occur at this time in relation to local lesions, resulting in severe inflammatory processes, and in general toxæmia. The increase in size of lesions is to be ascribed, at least in part, to the inflammatory reaction, and Debré, Paraf and Dautrebande noted that, in children, local lesions first became appreciable by physical or radiographic examination at the time when the cutaneous reaction was first obtained. The sudden onset of fever noted by Römer (1908) at the twelfth to fourteenth day after intravenous inoculation in cattle, and at the fifth to seventh day in sheep (1909^2) forms a part of the same picture, a picture which has obvious analogies, recognized by v. Pirquet, with the well-defined incubation periods of the infectious exanthemata.

From the theoretical standpoint, the most controversial questions are, perhaps, first, whether the development of allergy is a continuous or a discontinuous process, and, second, its relation to the formation of tubercle tissue. The delayed appearance of demonstrable allergy, and its rapid increase in degree at the time when it first appears, as well as the analogy noted with the infectious diseases of fixed incubation time, give apparent support to the conception of a latent period of elaboration ending with the fulminant appearance of sensitivity. But it has been shown that the interval can be varied within wide limits, according to conditions of dosage and other factors, while the rapidity with which allergy may appear after very large infecting doses suggests that its development is actually progressive from the time of infection. It is also to be recalled that the constancy of the incubation period in other infections may well depend on limits to the size of infecting dose possible under natural conditions. The question of the relation of allergy to the formation of tubercle tissue is discussed later, but it may be pointed out here that, while the development of sensitivity in the guinea-pig parallels, to a considerable degree, the evolution of lesions, the latter involves, as well as the increase in volume of tubercle tissue, a parallel increase in the number of bacilli present, and that these two factors cannot yet be separated experimentally.

On these two points the writer prefers, rather than assume the predominant importance of completely unknown processes, whose nature cannot be visualized to any extent, to adopt the simpler conception that allergy develops progressively, if not at a uniform rate, and that its development is controlled by quantitative factors, of which the most important is the extent of exposure of the tissues to contact with the specific protein of the tubercle bacillus. Such a generalization cannot, at present, be applied in detail to the complicated processes of infection, but the sense of it is that the variables noted—size of dose, virulence, path of infection, resistance of host—are of importance as determining the amount and rate of absorption of bacillary constituents.

Duration and variation of sensitivity.

For the maintenance of sensitivity, two conditions in particular seem to be important—that the general vitality of the host should not be seriously impaired, and that absorption of specific bacillary material should continue.

The influence of the first factor is seen in the decreased reaction intensity during intermittent periods of lowered vitality, and particularly during the final stages of the disease, when the cutaneous reaction may fail completely. v. Pirquet (1907^1) described the occurrence, in these conditions in man, of a peculiar type of skin reaction, delayed in appearance, cyanotic rather than bright red, and with little or no induration—the ' cachectic ' or ' torpid ' reaction. Diminished sensitivity of this type is probably due in part to the incapacity of the body to produce a high-grade inflammatory process, and Kraus, Löwenstein and Volk (1911) noted that when tuberculous guinea-pigs lost their capacity to react to tuberculin they no longer reacted to diphtheria toxin, although previously as sensitive to the latter as normals. There must also be kept in mind, however, the possibility of a process of desensitization by flooding of the body with reacting substance.

In the guinea-pig, it is generally held that sensitivity continues at a high grade until the final stage is reached, and this is well shown in the curves of Römer and Joseph (1910^2). Parish and Okell (1929) observed, in most of their animals, a decrease after the third or fourth week, but point out the deleterious effect on the animal of repeated testing and handling. This is a point of importance in experiments involving repeated skin reactions, and one the writer has frequently had occasion to note.

At the opposite end of the scale is the decrease in sensitivity which accompanies arrest of disease. The guinea-pigs inoculated by Krause (1916) with the avirulent strain, R 1, which produces localized lesions going on to healing, gave well-marked skin reactions up to 5 months, but, as the lesions diminished in size, the reactions became weaker. Willis (1928), using the same strain, obtained definite but weak reactions at 1 year, and at 2 years only occasional and indefinite effects with from 2 to 5 times the ordinary test dose of tuberculin. In man, the same effect may be seen with latency of disease, but the variations are irregular, and cannot always be correlated with the extent or course of infection. The fact that practically all adults in civilized communities react in some degree to tuberculin is frequently advanced as evidence of the rarity of complete healing, that is, of complete elimination of tubercle bacilli. Debré, Paraf and Dautrebande say that they have never seen complete disappearance of the v. Pirquet reaction in the healthy human subject.

In a third group, allergy may fail temporarily, often for a few days only. The best-known example is that of measles, in which sensitivity characteristically disappears completely during the eruption, the ' anergy ' lasting about a week (Preisich, 1907; v. Pirquet, 1908). Debré and Papp (1926) found that with the reappearance of sensitivity reactions developed at the sites of negative tuberculin tests made during the anergic period. In measles rendered benign by treatment with convalescent serum the reaction did not disappear.

A similar failure of reaction has been observed in influenza (Bloomfield and Mateer, 1919), in chicken-pox (Schonfeld, 1923), and in many other acute infections (Calmette, 1928[1]). Lelong and Rivalier (1923) found a large proportion of syphilitics negative during the acute secondary period. The reaction may fail during the *puerperium* (Nobécourt and Paraf, 1919, 1920), or during menstruation (Coulaud, 1921), while Paraf (1923) found that sensitivity disappeared in the tuberculous guinea-pig immediately after parturition, and that such animals tolerated an intracardiac dose of tuberculin which was fatal for tuberculous males. Decressac and Jacquelin (1924) noted failure of reaction after surgical operations under general *anæsthesia*.

In many of these conditions the factors are probably similar to those operating in the group of cachectic or terminal conditions, but the suddenness and constancy with which sensitivity disappears, for example in measles, suggests an effect more particularly related to the special mechanism of allergy.

Supersensitization.

The site of a local cutaneous or conjunctival tuberculin reaction frequently shows, when *tuberculin* is subsequently introduced into the circulation, a new inflammatory process, which may equal or exceed in intensity the original reaction (Klingmuller, 1903; Slatinéanu, 1907). This ' flare-up ' effect, in the case of the skin reaction, may also be produced by intracutaneous injection of tuberculin in the vicinity. Weiss (1925), on repetition of the v. Pirquet reaction at the same site, obtained reactions which developed at an increased rate, and, in the eye, a second instillation of the same dose of tuberculin may produce a reaction of increased intensity, or a positive reaction when the first test gave none (Cohn, 1907; Levy, 1908). v. Pirquet, with successive cutaneous reactions at fresh sites, noted a progressive increase in intensity of reaction. An increase of the febrile reaction with repeated subcutaneous injections has been described by Löwenstein and Rappoport (1904) and others. All these effects suggest that contact with *tuberculin* leads, in the allergic subject, to an increased sensitivity, local or general, a process of 'super-sensitization'. The effect is not clearly seen in the normal animal, and it must be assumed that the allergic organism has an increased capacity for developing sensitivity. This is also well demonstrated in the results of Willis (1928), in guinea-pigs inoculated with the avirulent strain, R 1. These animals, two years after injection, no longer gave a definite skin reaction, but on reinoculation with a virulent strain, sensitivity redeveloped so rapidly that necrotic reactions were obtained four days later, when normals receiving the same virulent dose were still negative. This process finds an analogy in the capacity of an animal which has once developed antibodies for a specific antigen, to redevelop these antibodies at an increased rate, after a fresh application of the antigen.

Tuberculin desensitization and tuberculin tolerance.

With the earliest use of the diagnostic reaction in cattle, it was recognized that subcutaneous injection of tuberculin in sufficiently large amount produced a decrease or abolition of sensitivity, so that a second test made within a few days might yield a negative result. The practice of thus 'doping' an animal so that it will pass an official test has proved a serious problem in veterinary work. In man, the cutaneous reaction may fail during the 2 or 3 days following a general reaction, though, according to Calmette (1928[1]), the conjunctival reaction may still be obtained.

The commonly accepted view is that the process is one of desensitization, and Borok and Pick (1926) have produced the effect locally by repeated application of tuberculin ointment to the skin, or by intracutaneous injection, the area being rendered insensitive to subsequent v. Pirquet or intracutaneous tests.

That the tuberculous subject may develop an increased tolerance to tuberculin with repeated increasing doses was stated by Koch (1890) in his original communication, and has formed the basis of most systems of tuberculin therapy. The degree of tolerance which can be produced in animals is very considerable. Burnet (1908), by treatment of tuberculous guinea-pigs with increasing doses of precipitated tuberculin, produced, in 40 days, a tolerance such that 100 mgm. could be given without serious effect, and Manaud (1909) reached doses of 200 mgm.—a dose of 20 to 30 mgm. being fatal for the untreated tuberculous guinea-pig. It is of interest that in Manaud's animals the tolerance rapidly disappeared on the cessation of treatment, and this strongly suggests that the effect was one of desensitization rather than of immunization.

There is, in fact, no evidence for any high degree of immunity against the toxic action of tuberculin in the allergic subject, in the sense either of active prevention of the reaction, or of neutralization of its toxic effects, and neither clinical nor experimental observation justifies, at present, any expectation of an efficient antitoxic serum. Pickert and Löwenstein (1908) believed that the serum of tuberculous subjects contained a substance ' anticutin ', which could neutralize the skin-reacting power of tuberculin, but subsequent attempts to confirm this have yielded most irregular results, and no clear confirmation has been produced. While a definite degree of tolerance develops in man, its limits are usually narrow, and cannot be exceeded even with prolonged treatment. Its mechanism is still obscure, and it cannot yet be decided to what extent it depends on desensitization, on active immunization, or on other factors.

Allergy without Infection.

The present position with regard to experimental production of allergy without infection is that it has been clearly proved that killed bacilli can produce typical sensitivity, but much less so that bacillary constituents in solution can have the same effect. The experimental work to be reviewed has a particular bearing on the highly controversial question of the dependence of allergy on tuberculous tissue, which will be considered in general discussion. It may be noted, however, that dead bacilli give rise to tissue lesions anatomically similar to those of infection, as first observed by Prudden and Hodenpyl (1891).

In many of the results described under this heading, and under that of *Specificity*, a type of skin reaction has been obtained which differs qualitatively from the typical reaction to intracutaneous tuberculin. These reactions have been emphasized and described by Zinsser and Tamiya (1926). While the induration plaque may be as large as, or even much larger than, the ordinary typical reaction, and may be sharply demarcated, it shows usually a diffuse erythema, and a soft, puffy œdema, rather than the firm, blanched condition of the typical plaque. Central necrosis may occur in the more intense grades, but is small in extent, and seldom reaches 10 mm. The reaction commonly reaches a maximum before the second day, and may be definitely fading at 48 hours. These reactions are definitely abnormal, and do not occur in the untreated animal, and in many cases they undoubtedly represent specific sensitization, but their significance is not, as yet, completely understood. They are referred to, in the following discussion, as ' atypical reactions '.

Sensitization by killed bacilli.

Krompecher (1900) obtained some febrile reactions in rabbits treated with dead bacilli, and Borrel (1908), by repeated subcutaneous injection of dead bacilli in rabbits, produced progressively larger abscesses with equal doses.

The first clear success in the production of skin sensitivity was obtained by Bessau (1916), who injected guinea-pigs with heat-killed bacilli by most possible routes, and, after 2 to 3 weeks, obtained strong intracutaneous tuberculin reactions, with many typical necrotic ' cocardes '. Intraperitoneal treatment gave the best results, while intravenous produced no clear sensitivity. Petroff (1923) treated guinea-pigs intraperitoneally with heat-killed bacilli, in doses of 12·5 to 37·5 mgm. (dry weight), and obtained skin reactions quite comparable with those seen in infection. Sensitivity appeared as early as the fifth day, more commonly about the tenth. Petroff and Stewart (1925) found that guinea-pigs sensitized by dead bacilli reacted in the same way as did tuberculous animals to the application of living bacilli to the skin, eye, pleural cavity or testis.

Lange and Freund (1926) produced similar results with intraperitoneal injection, and still better by multiple intracutaneous injections. Langer (1925) sensitized tuberculin-negative children by injections of heat-killed bacilli, intracutaneous or inunction reactions being obtained in 22 of 27 cases after 1½ to 3 months.

Wolff-Eisner (1922), Boecker (1923), Boecker and Nakayama (1923), and Nakayama (1924) have reported similar results in animals.

The writer has treated guinea-pigs with bacilli killed by heat, formaldehyde, or phenol, and obtained in every case marked skin reactions, usually necrotic. Small doses of bacilli appeared as efficient as large, and the largest reaction seen—necrosis 16 mm.—was in an animal which had received only 0·1 mgm. (dry weight). The reactions were qualitatively indistinguishable from those in tuberculous animals, and usually comparable in intensity.

Boquet and Nègre (1923[1], 1926) obtained clear febrile reactions in rabbits treated intravenously with heat-killed bacilli, as did Coulaud (1923), who found coarse emulsions more efficient than fine.

It might have been expected that a typical lethal reaction would not be obtained in animals sensitized by dead bacilli, since the factor of numerous disseminated focal reactions is lacking. Ungermann (1915) obtained a large number of fatal reactions on reinjection of bacilli, or of tuberculin, but the test doses were very large, up to 100 mgm. of bacilli, and some of the deaths were frankly anaphylactic. Crawford (1923), however, in a series of over 70 guinea-pigs, sensitized with heat-killed bacilli, constantly obtained typical tuberculin death within 24 hours after intraperitoneal injection of 0·25 c.cm. O.T.

In Crawford's animals sensitivity disappeared after about five months. Petroff (1924), with doses of 3·75 mgm. of killed bacilli, produced sensitivity lasting over a year, and believed the duration to be related to the persistence of the intraperitoneal pockets of bacilli and caseous material which are found after such injections.

Sensitization by tuberculin.

The earlier claims to sensitization of normal animals by treatment with tuberculin were made at a time when allergy and anaphylaxis were considered as identical, and most were frankly concerned with the latter process. The evidence for the production of allergic sensitivity by tuberculin is quite inconclusive, and most modern writers consider it as being entirely in the negative.

Calmette, Breton and Petit (1907) obtained some febrile reactions in rabbits on repeated intravenous injection, but did not consider them typical. A conjunctival reaction could be elicited in some of these animals at 16 hours after injection, and somewhat similar rapid sensitization of the conjunctiva has occasionally been described by others. The increased conjunctival sensitivity which Cohn (1907) and Levy (1908) obtained on repeated instillation in man were obtained in what were probably infected cases, as pointed out by Wolff-Eisner (1908) and have already been discussed as such. Wolff-Eisner says that the effect is not seen in non-tuberculous cases.

Mantoux and Perroy (1911) obtained intracutaneous reactions in guinea-pigs 10 days after subcutaneous injection of 0·2 to 0·5 c.cm. O.T., but other workers have failed to produce any typical skin reactions. Seligmann and Klopstock (1921) and Markert (1924) observed in tuberculin-treated animals occasional reactions which they compared to the Arthus phenomenon, and which appear to have represented the atypical form described above. The authors did not consider them as significant.

Sensitization by bacillary extracts and protein fractions.

Results with these materials, in which the bacterial protein may be present in larger amount, and possibly in less diffusible form, than in tuberculin, have been slightly more suggestive.

Petroff (1923), in guinea-pigs treated with extracts, and with the acid precipitate fraction, obtained some skin reactions, but they were inconstant, and did not occur when the injected solution was first filtered (Berkfeld), suggesting, as Petroff points out, that the results may have been caused by intact bacilli.

Zinsser and Tamiya (1926) had some success with extracts, but did not consider the results conclusive. Their reactions were mostly of the atypical form, and appeared only for a short period at about 4 to 6 weeks.

The writer has failed completely to produce sensitivity with bacillary protein in solution, but in a few animals injected with the dry protein, incorporated in beeswax, to give slow, continued absorption, strong skin reactions have been obtained. Some of these were almost characteristic ‘ cocarde ’ reactions, with considerable necrosis, while others were definitely of the atypical form. The stronger reactions showed a ‘ flare-up ’ on reinjection in the vicinity, and in one animal the skin over a subcutaneous wax and protein deposit, at 3 cm. distance from an intradermal tuberculin injection, showed a strong ‘ focal ’ reaction. It is to be noted, however, that reactions were only obtained in animals which had received at least one injection of the mixture intraperitoneally, and that this led to severe peritoneal inflammation, so that the results may be analogous to those discussed under the next heading.

Sensitization by exudates and tissue extracts from tuberculous animals.

McJunkin (1921) injected living tubercle bacilli intraperitoneally in tuberculous guinea-pigs, causing a fatal reaction, and injected the peritoneal exudate, after filtration (Berkfeld), subcutaneously in normals. These gave characteristic intracutaneous reactions, occasionally necrotic, after 8 to 10 days, the sensitivity disappearing after 1 to 4 weeks.

Paraf (1925^2) by the same method produced allergy ' identical with that following inoculation of living bacilli ', and lasting 3 to 4 months.

Zinsser and Tamiya (1926) injected tuberculin instead of bacilli in the tuberculous animal, and produced the same result with the filtered exudate.

Caspari (1925) obtained definite ' cocarde ' reactions in guinea-pigs injected with filtered extract of lung from a tuberculous guinea-pig, killed 2 hours after a small dose of tuberculin, but extracts of lungs from animals dying of tuberculosis had no effect.

Similar results have been obtained with extracts of tuberculous tissues, without preliminary injection of tuberculin or bacilli.

Nitschke (1924) perfused the lungs of tuberculous guinea-pigs with horse-serum, and injected the filtered perfusion fluid in normals, producing strong skin sensitivity of short duration.

Lange (1924) treated normal guinea-pigs with filtrates (Mandler) of extracts of subcutaneous tuberculous lesions from guinea-pigs, and, after a few days, obtained large skin reactions, but these were definitely of the atypical form. She obtained similar results with extracts of sterile inflammatory foci, produced in tuberculous animals by injection of kieselguhr, and Zinsser and Tamiya found the peritoneal exudates active when bouillon instead of tuberculin was injected in the tuberculous animal.

In many of these results there is a strong suggestion that the sensitizing power of the exudates or extracts depends on the occurrence of an inflammatory reaction, specific or non-specific, and that the bacillary protein, either injected, or derived from the tuberculous animal itself, is so altered by the inflammatory process that it acquires this sensitizing capacity. Most interesting, in this regard, is the recent work of Dienes (1928), who has sensitized tuberculous guinea-pigs to egg-albumin, by injection into tuberculous lesions, so that subsequent intracutaneous injection of this protein gave large, persistent, necrotic reactions, which seem to have been comparable with the tuberculin reaction.

Passive sensitization.

A few successful results in passive transfer of tuberculin sensitivity have been claimed, but have not received adequate confirmation.

Helmholz (1909) claimed that sensitivity could be transferred from a tuberculous to a normal guinea-pig by defibrinated blood, and by parabiosis (peritoneal suture), skin reactions appearing after 2 days in the first case and after 5 or 6 in the second. It is to be noted that his tests for sensitivity were made by the v. Pirquet method, generally found unsatisfactory even in the tuberculous guinea-pig. A number of attempts to confirm these findings have failed completely (Joseph, 1910; Neufeld and Dold, 1912; Calmette, 1928[1]; and others). Recently, however, Zinsser and Mueller (1925) believed that they were able to produce sensitivity in guinea-pigs with the serum of rabbits intensively treated with living tubercle bacilli, and they obtained strong skin reactions at 3 to 4 days after the serum injection.

Bail (1910) injected normal guinea-pigs intraperitoneally with emulsions of tuberculous guinea-pig organs—liver, spleen, lymph nodes—and about 24 hours later with O.T., usually 0·6 c.cm., by the same route. All animals developed severe reactions, usually fatal in 24 to 48 hours. Onaka (1910) obtained the same results in a small number of animals, but Joseph (1910), Kraus, Löwenstein and Volk (1911), Neufeld and Dold (1912), Selter (1921[1]) and Klopstock (1924) were unable to confirm them. It is to be noted that Selter's and Klopstock's protocols show some suggestion of the effect described by Bail, though the authors do not consider the results significant.

Specificity of the Tuberculin Reaction.

Within the tubercle bacillus group.

It is generally accepted to-day that no specific differences can be demonstrated, on the basis of the allergic reaction, between the human and bovine types of bacillus. A few claims have been made that in children, where bovine infection is prevalent, many cases react only (Klose, 1910; Synwoldt, 1920), or more strongly (Moro, 1920), to bovine tuberculin, but other workers have been unable to distinguish any differences corresponding to the type of infection, either in man (Gauvain, 1917; Nehring, 1923), or in animals (Kleinschmidt, 1918; Crawford, 1927). Bovine tuberculin (P.T.) frequently gives weaker reactions than human (O.T.), but this appears to result from the weaker growth in culture of the bovine bacillus. The quantitative relations between human and bovine tuberculin are the same when tested in animals infected with either type of bacillus.

As regards the avian bacillus, most results indicate a specific difference from mammalian types, particularly when quantitative factors are considered.

Fürth (1920) found that guinea-pigs inoculated with avian bacilli might give fatal reactions to intravenous injection of human bacilli (50 mgm.); and Boquet and Nègre (1926) say that a typical Koch's phenomenon can be obtained with human bacilli in animals repeatedly inoculated with the avian strain.

Crawford (1927), however, inoculated guinea-pigs with avian (4 strains), bovine and human bacilli, and tested them by subcutaneous and intracutaneous injection of avian and mammalian (human) tuberculins, and in all animals observed much stronger reactions to the homologous tuberculin. Many animals gave little or no reaction to the heterologous type.

Similarly, Jousset (1921) found that many human cases, reacting to O.T., gave no reactions to avian tuberculin, or, at most, no greater than to extracts of *B. coli* and other bacteria. He considered the effect as non-specific.

Within the acid-fast group.

The allergic reaction shows no close relationship between the tubercle bacillus and the 'cold-blooded group' of acid-fast bacilli from fishes, amphibia and reptilia, or the various acid-fast saprophytes from butter, milk, grass, &c. Nègre (1924) says that the non-pathogenic strains have little, if any, power to produce sensitivity to the tubercle bacillus, but that tuberculous subjects may react to these strains, or to 'tuberculins' prepared from them. The reactions, however, appear to be atypical, and their significance is probably the same as that of the reactions, described below, which are obtained with unrelated bacteria.

Fürth (1920) obtained occasional fatal reactions to intravenous injection of dead tubercle bacilli in guinea-pigs inoculated with turtle and grass strains, and Crawford (1926) describes atypical skin reactions to mammalian and avian tuberculins, after treatment with fish, smegma, and 'hog-skin' bacilli. Boquet and Nègre (1926) found that guinea-pigs repeatedly treated with 'paratubercle' bacilli reacted to the intracutaneous injection of tubercle bacilli with infiltration and necrosis, but were resistant to large doses intraperitoneally.

Meyer (1923), in children injected with 'cold-blooded' strains, obtained skin reactions to the homologous 'tuberculins' but not to O.T., but tuberculous children reacted to all the 'tuberculins'.

In tuberculous guinea-pigs, only fleeting, atypical skin reactions are produced by the non-pathogenic 'tuberculins' (Sons and Mikulicz-Radecki, 1921; Lange and Lange, 1922; Scheff, 1925).

Selter (1920) obtained no reactions on intravenous injection of turtle bacillus 'tuberculin' (Friedmann), and Dietrich (1921) obtained fatal reactions only with large doses. Uhlenhuth and Lange (1920), however, report typical tuberculin death in tuberculous guinea-pigs injected with turtle 'tuberculin', intraperitoneally (0·5 c.cm.) or subcutaneously (1·0 c.cm.). Long (1924), and Long and Seyfarth (1924) obtained 'mild but definite' testicular reactions in tuberculous guinea-pigs with turtle, frog, grass and smegma bacilli.

In man, most workers have failed to obtain v. Pirquet reactions with these various 'tuberculins'. Some intracutaneous reactions are described, but Lange and Lange found the threshold dose to be from 10 to 1,000 times as high as for O.T. Selter and Tancré (1924) describe reactions to turtle and milk bacilli in tuberculous subjects which, however, were weaker than those to *B. coli.*

Dunkin (1927) found no confusion between the reactions to 'Tepsin' or 'Johnin' in cattle infected with the 'warm-blooded' Johne's bacillus, and those to tuberculin.

With relation to bacterial and other proteins.

It has been frequently stated, in recent years, that in tuberculosis, and particularly in man, there is an increased and abnormal sensitivity to foreign proteins of the most varied type. There is considerable evidence to support this view, but very little for the claim, occasionally made, that this process is the entire basis of the tuberculin reaction. The evidence so far produced, while of some theoretical interest, suggests, at most, that non-specific factors are involved in the allergic reaction, but does not affect the position of the orthodox view that certain highly specific factors are essential.

Sorgo (1911) claimed that diphtheria, dysentery and cholera toxins gave skin reactions which were parallel, both in incidence and in intensity, with the tuberculin reaction. This claim was attacked by Zieler (1911), who found that the reactions to dysentery and typhoid culture filtrates, while often large, were atypical and rapidly fading, while their incidence was not strictly parallel with that of tuberculin sensitivity, and that the sites did not give ' flare-up ' reactions with subcutaneous tuberculin injection. Hollo and Amar (1921) and Zieler and Markert (1922) found only a false parallelism between the tuberculin and the toxin reactions, and the former obtained reactions to diphtheria toxin with the same frequency in non-tuberculous as in tuberculous children. Selter (1922[1]) produced reactions to *coli* and dysentery emulsions both in tuberculin-negative and in reacting cases, but in the latter they were more persistent. Selter and Tancré (1924) produced strong skin reactions with *coli* emulsions in tuberculous patients, often larger at 24 hours than those with tubercle bacilli, but at 48 hours the specific reactions were increased, the non-specific much decreased. Subcutaneous tuberculin produced ' flare-ups ' at both sites, *coli* injections at neither. Fernbach (1926) was unable to obtain ' flare-ups ' at non-specific reaction sites.

Blumenberg (1925) found that the sites both of *coli* and of tuberculin reactions developed ' tuberculoid ' tissue—an interesting commentary on the ' specific ' nature of this tissue. Zieler and Hämel (1926) obtained strong *coli* reactions in tuberculin-negative cases, and often none in those highly sensitive to tuberculin. ' Flare-ups ' with subcutaneous tuberculin were seen at *coli* sites only when a general reaction occurred. They cite an interesting case with combined tuberculosis and chronic glanders. Skin reactions were given both by tuberculin and by mallein, but subcutaneous injection of either produced ' flare-ups ' only at the homologous sites.

A separate controversy arose from the results of Schmidt and Kraus (1919), Schmidt (1920), who found that subcutaneous injection of foreign proteins—particularly milk—in tuberculous patients, gave general and focal reactions as seen with tuberculin. They advocated the therapeutic use of milk in place of tuberculin, and a considerable literature has arisen about this ' proteinkorpertherapie '. It has been found generally that relatively large doses are required to produce reactions, and that milk or milk proteins give very indefinite skin reactions (Sons and Mikulicz-Radecki, 1921 ; v. Friedrich, 1921 ; Selter, 1922[1]). The results of Epstein (1921) are important in this regard. He found that only the milk of tuberculin-reacting cows gave reactions in tuberculous patients. The milk from one negative cow at first did not elicit reactions, but a few months later did so, and it was then found that the cow had become a reactor in the interval.

In attempting to explain the non-specific reactions in general, three main possibilities may be suggested. (1) That the effect is quite independent of tuberculin sensitivity, and any apparent parallelism accidental. The data obtained from the use of the Schick reaction, for example, would seem adequately to explain the reactions to diphtheria toxin, and it is possible that some of the reactions to other bacteria are of the same type, i.e. a primary toxic effect, to which a proportion of the population have become immune. Or the reactions may represent independent specific sensitivities of the type seen in asthma and hay fever. (2) That in tuberculosis there is an increased sensitivity to irritants in general. (3) That the development of sensitivity to a specific bacterial protein involves some increased sensitivity to bacterial proteins in general, or an increased capacity to develop such sensitivity. The results of Zinsser and Tamiya (1926) with *B. abortus* definitely suggest an ' overlapping ' of the reactions to bacterial proteins.

Selter, a persistent advocate of the view that *tuberculin* allergy involves a non-specific increased irritability of allergic tissues, admits, however (1926), the possibility that the reactions of the tuberculous subject to other bacteria may represent pre-existent specific sensitivities, increased by the tuberculous infection. He does not attack the quantitative specificity of the tuberculin reaction as a practical test. The quantitative relations, in fact, between the effect of tuberculin in the tuberculous subject, and that of non-specific protiens, appear to be quite of the same order as those between specific and non-specific serological reactions, and there is, at present, no reason to doubt the specificity of the tuberculin reaction as a diagnostic method. This has been confirmed in world-wide clinical use, and by post-mortem findings, to a greater degree, perhaps, than for any other biological test.

Elimination of Tuberculin.

Injected *tuberculin* is rapidly eliminated by the kidneys, both in normal and tuberculous animals, and the rate is not greatly different in the two cases.

Franceschelli (1914) injected tuberculin intravenously in normal rabbits, and tested the urine by intracutaneous injection, in serial dilutions, in tuberculous guinea-pigs. By comparison with known dilutions of tuberculin he estimated that the injected *tuberculin* was recovered quantitatively, and that elimination was complete at 6 hours, almost so at 3 hours.

Frisch and Klimesch (1924) in normal, and Frisch and Silberstern (1924) in tuberculous rabbits, found that *tuberculin* injected intravenously rapidly disappeared from the blood, which gave good reactions up to 2 hours, weak reactions up to 12 hours, and none after that. The urine from tuberculous animals collected in the first 3 hours gave strong reactions, that from the second 3 hours much weaker. Ornstein and Steinbach (1925) obtained similar results in normal and tuberculous guinea-pigs.

Holst (1921, 1922) found that *tuberculin* disappeared very rapidly from the blood of normal rabbits, but obtained weak reactions with the urine, and believed that the active substance was fixed for a time by certain tissues.

Wildbolz (1919) with the concentrated urine of patients with active tuberculosis, injected intracutaneously in the same case, obtained reactions which he considered to be specific, and great interest was aroused in the method as a diagnostic test. It has yielded, however, most discrepant results, and general opinion is that the method, at least in its original form, is not reliable. Enright and Rettger (1924), however, who dialysed the concentrated urine and injected it in cases of known sensitivity, obtained reactions with every urine from 30 tuberculous patients, and with none from 16 non-tuberculous cases.

While it must be assumed that what specific bacillary substance reaches the blood from tuberculous lesions will be excreted in the same way as injected *tuberculin,* the degree of febrile reaction in the majority of patients does not suggest that it is continually present in the circulation in such amounts that it could readily be detected in the urine by the methods so far employed. A diagnostic test which will be generally applicable will require a method which permits of concentration of the active material to a much greater degree.

The rapid excretion of *tuberculin,* as compared with that of serum-proteins or egg-albumin, which can be detected in the blood, by serological tests, for at least several days after injection, has two points of theoretical interest. It suggests that the molecule of the active substance may be of smaller size than that of the well-known animal proteins, and the characteristics already described—questionable heat coagulation, low nitrogen content—are evidence in the same sense. Again, it offers a possible explanation of the failure to sensitize with tuberculin or bacillary extracts, and this view is supported by the results of Zinsser and Mallory (1924), who found that anaphylactic sensitization with bacterial proteins required large and repeated doses.

Allergy and Anaphylaxis.

v. Pirquet used the term *allergy* for a ' clinical conception ', covering all forms of altered reactivity, without commitment as to the mechanism, but obviously regarded the altered reactivity of microbic infection, typified by the tuberculin reaction, and that of protein anaphylaxis, as representing closely related processes. The confusion of the two conditions is evident in most of the early work on the effects of tuberculin (see Doerr, 1913), but subsequent investigations, particularly those of Baldwin (1910), and Krause (1911), showed that anaphylaxis can be produced by the protein of the tubercle bacillus, and without the occurrence of tuberculin sensitivity as seen in infection. Baldwin and Krause sensitized guinea-pigs with bacillary extracts, and elicited typical anaphylactic reactions, and the picture was completed for passive transfer by Austrian (1913), Austrian and Fried (1913). Weil (1917) showed further that anaphylaxis may develop in the course of infection, and by the Schultze-Dale method, with the uterus of tuberculous guinea-pigs, obtained typical contractions on contact with tuberculin.

Baldwin found, however, that the characteristic tuberculin reaction occurred only with infection, and recognized that it probably depended on a separate process. This latter view received increasing acceptance from that time, and its accuracy was clearly demonstrated by Zinsser (1921), who showed that anaphylaxis and *tuberculin* allergy might, but did not necessarily, co-exist in the infected animal. Tuberculous guinea-pigs gave strong skin reactions before the uterus showed any evidence of anaphylactic sensitization. Zinsser also described the occurrence, in the guinea-pig, of a type of skin reaction which is associated, in man, with anaphylaxis, and particularly with the sensitivity of hay-fever and asthma—a rapidly developing œdema, disappearing in the course of an hour or two, without tissue injury. He contrasted this with the typical tuberculin reaction—delayed in appearance, persistent, and definitely inflammatory in nature, often causing extensive injury.

That the two types of sensitivity involve separate mechanisms is to-day accepted by most authorities without reserve, and the distinction is commonly made according to the following more or less constant formula.

Anaphylactic sensitivity is produced by injection of protein in solution, its development is accompanied by a production of antibodies of the *precipitin* type, and normal animals may be passively sensitized by injection of serum from sensitive animals. Intracutaneous injection of specific antigen, in the sensitive animal, produces a rapidly appearing, temporary œdema suggesting a vasomotor effect. Intravenous injection, in the guinea-pig, leads to rapid death, with characteristic distension of the lungs due to spasm of the bronchial musculature, while the effect on smooth muscle may also be demonstrated on the isolated uterus or intestine.

Tuberculin allergy in characteristic form has been produced, with some exceptions whose significance is as yet uncertain, only by infection or by more or less intact bacilli. Antibody development does not seem to be closely associated with the occurrence of the allergic reaction, and the evidence for passive transfer of sensitivity is indefinite, and generally considered as negative. Cutaneous application of *tuberculin* leads to a delayed, persistent reaction of inflammatory nature, and the entry of *tuberculin* into the general circulation produces a febrile and toxic reaction which is only fatal after some hours, and is associated with focal inflammatory lesions. The condition may exist without any evidence of muscular sensitization.

It remains to be said that the more recent work has already cast some doubt on the validity, in any absolute sense, of some of these generalizations, and particularly of that concerning the production of sensitivity. It is also to be noted that the characteristics of the two types of reaction, as described, are based almost entirely on observations in the guinea-pig and in man. It has been pointed out by Zinsser (1924) and by Opie (1924) that in rabbits sensitized by egg-albumin, or by serum-protein, the anaphylactic skin reaction is very similar to the tuberculin reaction in that animal—a delayed inflammatory process, often necrotic. It is clear, then, although the allergic reaction is characteristic for microbic infection, that the production of an inflammatory reaction, as such, is not peculiar to bacterial proteins. The tuberculin reaction in the rabbit, however, is not highly characteristic, and the similarity of the two reactions in this animal does not nullify the importance of the plain dissimilarity in the guinea-pig, as indicating the existence of two distinct mechanisms. It is difficult to form any rational opinion as to whether the development of the one or the other type of sensitivity depends on differences in the nature of the antigen, or in the conditions under which it acts, and it is quite impossible, as yet, to decide whether or not the two are based on any common fundamental process. The obvious difference lies in their manifestations and the characteristic feature of the allergic reaction is its inflammatory nature. The evidence suggests that this quality is a primary rather than a secondary one, and it seems probable that allergic sensitivity will be found to be a conditioning factor in most types of bacterial inflammation.

Discussion.

There remain for discussion the general problems concerning the process by which allergic sensitivity is produced, and the mechanism of the reaction.

For the production of allergy, perhaps the most widely stated opinion is that it depends, in some way, on the formation of tubercle tissue. This idea rests largely on negative evidence, the failure, so far, to produce typical sensitivity by any method which does not also involve the formation of the anatomically characteristic tissue. If this view is taken, as it appears to be in some quarters, as meaning that the essential reacting factor is produced only by the cells of tubercle tissue, it has to meet certain serious objections.

In the first place, it is most improbable that the development of anatomical tubercle is a direct response to the specific bacillary substance concerned in the allergic reaction. It is a type of tissue characteristic for the effect of insoluble irritants, particularly those of fatty nature. Ray and Shipman (1923), by injection of chloroform extracts of tubercle bacilli, have produced typical epithelioid and giant-cell formation, not only locally, but in regional lymph nodes ; and Krause (1927), one of the most persistent supporters of the dependence of allergy on active lesions, accepts tubercle tissue as, in the first place, a response to bacillary wax. Medlar (1926), however, supported by Stewart and Rhoads (1926), thinks it is in part a response to necrotic tissue, which, in its turn, may be the result of an inflammatory allergic reaction. This would make tubercle tissue the result, rather than the cause of allergy, but still, in itself, a non-specific process.

Again, there can be little doubt that, in the tuberculous animal, a local tuberculin reaction can be elicited in any of the body tissues. There is no reliable evidence that the reaction is due to serum-borne antibodies, and it must be assumed, for the present, that the process is related either to the fixed tissues or to circulating cells. That the leucocytes may play an important part is suggested by their constant presence in the acute phase of the reaction, while the results of Stewart, Long and Bradley (1926) indicate that these cells may, themselves, be sensitized to *tuberculin.* In the purulent pleural exudate produced by injection, in the allergic guinea-pig, of bouillon *plus* tuberculin or bacilli, the leucocytes were all dead at 24 hours, while in the normal controls they were all living. On the other hand, it is difficult to explain the local increase of sensitivity which follows the application of *tuberculin* in the allergic animal, save on the ground of sensitization of fixed tissues. It seems probable, then, that the process of sensitization affects all tissues of the body, and if it depends on some factor of the nature of an antibody, produced only in tuberculous lesions, it remains to be explained how this factor is distributed to the tissues without being demonstrable in the blood.

It seems, moreover, unnecessary to assume the existence of any process more complicated than an active sensitization of the tissues generally, by exposure to a specific antigen derived from bacilli present in the lesions. The higher degree of sensitivity of the tissues surrounding the lesion would be explained, on this basis, by the higher concentration of antigen. That there is a considerable degree of parallelism between the formation of tubercle tissue and the development of allergy is undoubted, but, in the writer's view, this can be explained on the basis of common mechanical factors. *Tuberculin* in solution is rapidly eliminated, and it is probable that sufficient exposure to produce sensitization can only be obtained where there is continued absorption of antigen, as is the case when bacillary matter is present in particulate form. This, however, is also the condition necessary for the formation of tubercle tissue, though the two processes seem to depend on different chemical factors. There is, in addition, the probability, strengthened by recent work, that sensitization is influenced by the effect of an inflammatory process as such, in the sense of an effect on the form in which the antigen is distributed to the body. This view has already been mentioned in connection with sensitization by exudates and tissue extracts.

As regards the mechanism by which the allergic reaction is brought about, the outstanding fact to be explained is that *tuberculin*, relatively innocuous for normal tissues, is highly toxic, in minute doses, for the tissues of the allergic animal. The broad explanation which is generally accepted is that which represents the central idea of the schematic theories of Nicolle (1908) and of Wolff-Eisner (1908), that, from the contact of allergic tissue and *tuberculin*, a toxic substance is produced. This is, indeed, the only rational theory which can be formulated in terms of present biochemical knowledge. Selter's view, that allergic tissues are abnormally irritable, merely restates the problem, and evades the question of specificity, as does also Neufeld's (1921) suggestion of an increase of normal protective forces. Selter, however, logically enough demands the demonstration of a toxic product, and of the disappearance of *tuberculin* during the reaction. Neither Selter (1921[1]) nor Long and Seibert (1926[2]) were able to show any toxicity for normal animals, or any loss of toxicity for allergic animals, in mixtures of tubercle tissue and tuberculin. But no significance can be placed on such failure until we know more of the conditions governing the reaction, and particularly of the quantitative factors. As Zinsser and Tamiya (1926) point out, the evidence suggests that the reaction takes place in intimate relation to allergic cells, if not intracellularly. It may well be that a toxic substance is produced which is labile, or which represents only a phase in a complicated reaction, and which may act only on the cell in which it is formed. There is, however, some suggestion, in the febrile reaction, in the histological picture of local reactions, and in the reactions obtained by Zinsser and Tamiya in normal animals with mixtures of tuberculous tissue and tuberculin, that a free toxic body actually occurs. The failure to demonstrate a disappearance of *tuberculin* means little when it is recalled that the smallest concentration difference distinguishable by animal tests is of the order of 30 per cent. If the reaction is intracellular, the proportion of *tuberculin* which enters the cells and is destroyed may be very much smaller than this.

But, while it seems necessary to assume some primary specific chemical reaction, which analogy with known enzyme action suggests might cause the formation of a toxic degradation product of *tuberculin*, it is by no means certain that the inflammatory process, which is the most evident and characteristic feature of the allergic reaction, is in itself a specific one. The anatomical characters are those of inflammation in general, including reactions in which there can be no question of specific sensitization. The general or local toxic effects may well be secondary and non-specific, and it is to be noted that recent work indicates that a histamine-like substance may be liberated wherever there is cell injury. No sharp line can be drawn, at present, between specific and non-specific inflammation, but it is difficult to avoid the conclusion that, in the former, the specific factors must operate during a limited phase. Zinsser and Tamiya have made a suggestion which is attractive in this regard, that there is a specific fixative factor which establishes contact between *tuberculin* and the allergic cells. Such a factor might well be related to the well-known humoral antibodies, and would account for the specificity of the tuberculin reaction, though being only a phase in the complete reaction. It would still seem necessary to assume an increased reacting power on the part of the cells.

Passing to more superficial aspects, the conditions obtaining at the site of tuberculous lesions claim some attention. We have, on the one hand, the intense focal reactions which take place following doses of tuberculin which can give only a most minute concentration in the blood, and, on the other, the apparent anomaly that these highly sensitive tissues, in proximity to a focus containing bacilli, are not in a constant state of reaction. Both of these conditions are probably connected with the walling-off of the bacilli by a relatively avascular tissue. What quantitative data we have do not exclude the possibility that a focal reaction is a reaction simply to circulating *tuberculin*, but clinical observations suggest that any effect which produces increased vascular activity can lead to a mobilization of *tuberculin* from the disease focus, resulting in a reaction in the surrounding tissues. On this view, either a minimal reaction to circulating *tuberculin*, or the effects of over-exertion, of passive congestion, or of non-specific irritants, may have a ' trigger ' action, which will lead to a strong focal reaction.

Spontaneous reactions, occurring as a result of diffusion of *tuberculin* from the lesions, are generally accepted as the cause of many symptoms, but neither clinical data, nor the examination of post-mortem material suggests that the process is continuous, and here, again, the avascular tissue barrier is probably responsible. Medlar (1926) believes that every tubercle goes through a stage of acute inflammatory reaction, presumably allergic, with a heavy influx of leucocytes, which cause the central necrosis, and that this is followed by a delineation of the central zone, and by giant-cell formation at its borders, after which the acute inflammation subsides. The acute reaction stage is well seen in guinea-pig tissues, but is less definite in man, where the lesions available for study are commonly of longer duration, while post-mortem material will usually give the picture of the terminal ' anergic ' stage.

The general febrile reaction is commonly considered as secondary to the focal reaction, presumably as a result of toxic substances produced. Experimental support for this is given by the results of Trudeau, Baldwin and Kinghorn (1904), Klemperer (1914), and Toenniessen and Friedrich (1926), which indicate that, in the earlier stages of infection, excision of the primary tuberculous focus is followed immediately by a failure of the general reaction to tuberculin. And in the tuberculous guinea-pig which has died from a dose of tuberculin, the focal changes seem sufficient to account for death, even from non-specific effects. Theoretically, however, it is to be expected that the sensitized tissues of the body generally will take part in the reaction, and the fatal reactions obtained by Crawford (1923) in guinea-pigs sensitized with dead bacilli strongly support this view.

The results described under the head of specificity are very difficult to evaluate, and it is by no means clear that the atypical reactions obtained from non-specific materials are closely related to the tuberculin reaction. They offer, however, an interesting field for speculation, as suggesting that in allergic sensitivity there may be a non-specific increase of capacity to react to bacterial and other proteins in general, together with a specific fixative factor as suggested by Zinsser and Tamiya.

Space does not allow discussion of bacterial allergy in general, but it may be noted that reactions essentially similar to the tuberculin reaction occur in many different infections, while there is considerable evidence to suggest that the inflammation produced even by such bacteria as the staphylococci and streptococci, usually considered as inherently 'pyogenic', may be largely the result of an allergic process.

IMMUNITY.

The Manifestations of Acquired Immunity.

The experiments by which Koch (1891[1]) demonstrated the allergic reaction in the tuberculous guinea-pig furnished also the first clear experimental proof of an acquired immunity in tuberculosis, although its existence was already suggested by earlier study. The normal guinea-pig, inoculated subcutaneously with living tubercle bacilli, showed no immediate lesions, but in 10 to 14 days developed a nodule which broke down to form a persistent tuberculous ulcer. The same inoculation, in the tuberculous animal, produced the acute necrotic reaction already described, but the ulcer which followed the reaction healed rapidly, and the lesion disappeared without the development of gross tubercle tissue either at the site or in regional lymph nodes. These observations were followed by years of controversy, during which there was a general failure to confirm either the occurrence of the necrotic reaction or the resistance to reinfection, and it was only after nearly two decades that the accuracy of Koch's description was fully recognized and the conditions governing the phenomena realized.

The resistance of the tuberculous animal to reinfection, confirmed by della Cella (1904) and Detre-Deutsch (1904), was extensively studied during the following years, and the work of this period, culminating in that of Römer (1908, 1909[2]) and Römer and Joseph (1910[1 & 2]), established particularly the overwhelming importance of quantitative factors. It showed that, while a small dose of virulent bacilli might produce no appreciable infection in the already tuberculous animal, a large dose might give extensive lesions, while intermediate doses, in general, gave lesions of more limited extent and of more chronic type than in the normal.

The observed effects vary considerably with the route of reinoculation and will be considered under separate headings.

Cutaneous and subcutaneous reinoculation.

While the earlier experiments showed rapid healing of lesions at the site of subcutaneous reinoculation, the descriptions are not, as a rule, extensive enough to indicate whether the immunity was complete. A complete absence of tuberculous lesions following reinoculation in the guinea-pig was demonstrated by the application of bacilli to the intact skin (Courmont and Lesieur, 1908), or to the lightly scarified skin (Hamburger, 1909, 1910), both methods involving extremely small infecting doses, though sufficient to produce disease in normal controls. Römer (1908, 1909[2]) and Römer and Joseph (1910[1 & 2]), injected subcutaneously weighed doses of bacilli, down to 0·000,000,01 mgm. (moist), a dose which, according to present data, should average less than one bacillus, but which, in the experiments, gave progressive disease in normals. They obtained complete absence of reinfection in guinea-pigs only with the smaller doses—from 0·0001 mgm. to 0·000,000,1 mgm., according to the strain of bacillus—but with larger doses there was still marked evidence of resistance as compared to normal controls. In tuberculous sheep, complete immunity was observed to subcutaneous reinoculation of 0·2 mgm. bovine tubercle bacilli per kilogram body weight.

With reinfecting doses sufficiently large to produce local lesions, there is an interesting variation in the type of lesion, according to the conditions of dosage and of resistance. It is to be recalled that intracutaneous or subcutaneous inoculation, in the normal guinea-pig, gives a nodule at 8 to 10 days, which ulcerates at 2 to 3 weeks, the ulcer persisting to death, while the regional lymph nodes and the internal viscera are progressively involved. The effects described in the tuberculous animal fall into three general classes: (*a*) The acute inflammatory, necrotic reaction—Koch's phenomenon—followed by ulceration and rapid healing; (*b*) acute abscess formation within the first few days, going on to persistent ulceration; (*c*) the formation of a nodule similar to that of primary infection, but which develops much more rapidly, and may show no ulceration, or ulceration with subsequent healing—an accelerated and abortive lesion.

Koch's phenomenon requires a high degree of allergic sensitivity, and, apparently, a substantial reinoculation dose. It has usually been described as followed by complete healing, but there are few records of observation sufficiently prolonged to exclude a delayed development of lesions. Bezançon and de Serbonnes (1914) and Rolland (1914) found that it was not obtained either in the early stages of infection or in advanced disease. Acute abscess formation appears to represent a definite but lower degree of allergy. Bezançon and de Serbonnes obtained it with early or late reinoculation, but found that, in the same animal, a large dose would give the necrotic reaction, a smaller dose an abscess, while even in fully sensitive animals an abscess could always be produced by reducing the dose. These results strongly suggest a destruction of bacilli during the allergic reaction, provided the latter is of sufficient intensity.

The accelerated formation of a characteristic lesion has, perhaps, a closer bearing on natural reinfection, and a less clear relation to allergy, than have the first two types of reaction. Debré and Bonnet (1922) inoculated guinea-pigs intracutaneously every 5 days with 0·1 mgm. of bacilli, and found that the third and subsequent inoculations gave rapidly developing nodules, which did not ulcerate. With a 10-day interval, the second and third doses gave this same type of lesion, the fourth and fifth either the necrotic reaction, or sometimes an abscess. The accelerated lesion formation is beautifully illustrated in the experiments of Krause and Peters (1920). Normal guinea-pigs, injected intracutaneously with 'a few hundred' virulent bacilli, showed nodule formation during the second week, with ulceration during the third week. The nodules persisted to death, with slow increase in size, and with the formation of secondary tubercles about the ulcer. The same dose, in animals previously inoculated with the avirulent strain R 1, was followed by a light allergic reaction at 24 hours, rapidly subsiding. Nodules appeared at 4 days, and were well established at a week. The maximum size was reached at 2 weeks, and ulceration did not appear, as a rule, until the fourth or fifth week, and was sometimes absent. It was small in extent, and the lesions went on to healing, practically complete at 8 to 9 weeks.

Intravenous reinoculation.

Intravenous reinoculation has been studied chiefly in the larger domestic animals, particularly in cattle, and is of interest from the striking degree of resistance which may be shown by animals bearing only insignificant lesions, or immunized by a primary infection which has produced no permanent lesions, to a dose of bovine bacilli which is rapidly fatal for the normal. After a primary intravenous inoculation of such bacilli in cattle, or in sheep, there develops, after an initial incubation period, an acute febrile disease, fatal at from 3 weeks to 2 or 3 months, with an extreme grade of generalized miliary tuberculosis.

v. Behring and his associates (1902) showed that cattle could be immunized by treatment with human tubercle bacilli so that they completely resisted, at least for a period of some months, a subsequent intravenous inoculation with bovine bacilli. Neufeld (1903) obtained similar results in asses, goats, and cattle, and Koch, Schutz, Neufeld and Miessner (1905) produced striking results in calves immunized by intravenous injection of freshly isolated human strains. Fifteen calves were reinoculated intravenously, 3 months after the immunization, with a dose of bovine bacilli at least 4 times that fatal, in 1 month, for normals. Eight of these, autopsied 119 to 236 days later, were completely free from tuberculosis, and 2 showed only limited pulmonary lesions. The other 5 were alive and healthy at similar periods. Similarly, in sheep bearing localized subcutaneous lesions, Römer and Joseph (1910[1]) obtained complete absence of other lesions at 10 months after an intravenous inoculation of bovine bacilli which killed normals in 2 months. Calmette and Guérin (1908) found that cattle infected primarily by the digestive tract showed similar resistance to intravenous reinoculation, but that lesions might develop from the second infection after a period of months.

Lesions resulting from intravenous reinoculation in artificially or naturally infected cattle were always of a chronic form, and contrasted sharply with those occurring in the normal controls.

Debré and Paraf (1920) found that tuberculous guinea-pigs, reinfected by intracardiac injection, lived in most cases as long as non-reinfected controls—250 to 350 days—while normals infected by the same method died in 16 to 45 days.

Intraperitoneal reinoculation.

Reinoculation in the peritoneum of the tuberculous guinea-pig is followed by a severe allergic reaction, with abundant purulent exudate, but if the animal recovers from the acute reaction there appears to be a high resistance to the reinfection. Manwaring and Bronfenbrenner (1913), after reinoculations of 400,000,000 bacilli—about 10 mgm. moist weight—found, 4 or 5 weeks later, few or no peritoneal tubercles save on the omentum. Rist, Léon-Kindberg and Rolland (1914[1]), with a similar reinoculation dose found, in 6 animals which recovered from the acute reaction, no trace of peritoneal tubercles at 10 days to 2½ months.

Intrapulmonary reinoculation.

The effects of reinoculation by inhalation, or by intratracheal injection, have given somewhat discordant results, which do not give much clear evidence on the problem of immunity. This has obviously been due, where the dosage was large, to complication of the picture by the effects of the acute allergic reaction. All who have studied the method, however, describe a difference between the lesions of primary inoculation by this route, and those of reinoculation. Bezançon and de Serbonnes (1914) found that the lesions of reinoculation showed less caseation, and more tendency to proliferative changes and to fibrosis, while bacilli were much less numerous than in primary lesions. Baldwin and Gardner (1921), in extensive experiments, obtained scantier lesions, with less caseation, from reinoculation. Others, as Philibert and Cordey (1925), Arloing and Dufourt (1924), have obtained extensive caseation and cavity formation from reinoculation, but the doses appear to have been large, producing extensive necrosis from the allergic reaction. Grysez and Petit-Dutaillis (1912) and Grysez (1913) noted a relationship between the occurrence of large caseous lesions and the interval between inoculations, these lesions being more pronounced with longer intervals. With a few exceptions all these experiments were carried out in guinea-pigs.

Methods of Immunization.

It has been generally held that the highest degree of resistance to reinfection occurs during the course of a primary infection which causes progressive, or at least active disease, and Römer (1908) believed that immunity was greater the more virulent the primary infecting strain. On the other hand, it has been found by most workers, including Römer, that a severe primary infection, in the guinea-pig, rendered it difficult to obtain clear proof of immunity, both from confusion of the lesions resulting from primary and secondary infections, and because the animals rapidly reached the terminal stage where both allergy and immunity were diminished. The most satisfactory results, in this animal, have been obtained after primary infection, either with strains of low virulence, or with very small doses of virulent strains, the effect, in either case, being to produce a disease of slowly progressive type.

For practical preventive immunization, the production of progressive disease is excluded, and a method must be employed which produces lesions of limited extent, and which are neither progressive nor capable of becoming so at a later date. It is also important that animals should not, through the immunizing dose, become a source of infection for man. In the numerous attempts to attain these conditions, in cattle and in man, a number of methods have been employed which differ in principle, and may be considered separately. For comprehensive reviews of the subject the reader is referred to Löwenstein (1913) and Calmette (1927, 1928[1]), and for the earlier work in cattle to Zwick and Titze (1913).

Immunization by heterologous strains.

The attempt to apply the principle of Jennerian vaccination to tuberculosis was first made with the avian bacillus in cattle. Only a low degree of immunity seems to have been produced, but more recently MacFadyean, Sheather, Edwards and Minett (1913) have had somewhat better results. When the human and bovine types of the bacillus were differentiated, the use of the former, shown to be of low virulence for cattle, appeared to promise successful immunization in this species, and the method was widely examined, both in the laboratory and in veterinary practice.

The most important methods were those of v. Behring, and of Koch and his co-workers, already mentioned. v. Behring's ' Bovovaccine ' was prepared from a human strain cultivated in the laboratory for six years. It was first used as a fresh emulsion, but later was dried before use. ' Tauruman ', prepared by Koch and Schütz, was an emulsion of freshly isolated human strains. Both preparations were injected intravenously in calves. Bovovaccine was widely used in practice, and was the subject of several organized tests (see Vallée and Rossignul, 1906). It was found that a definite immunity to reinfection by the bovine bacillus was produced but that this was of short duration, in general, a year or less. Similar results were obtained with Tauruman (Weber and Titze, 1907, 1908; Weber, Titze and Jorn, 1910). Further, not only was the duration of immunity insufficient for practical prevention of bovine tuberculosis, but it became evident that, while the injected human strains caused no significant lesions in cattle, the bacilli were demonstrable in the tissues for several months, and that the milk of immunized cows might contain living tubercle bacilli for much longer periods (Griffith, 1913). The method was soon abandoned.

Claims have been made for successful immunization or treatment, in man, by the use of acid-fast bacilli of the ' cold-blooded ' group. Of these claims, those of Friedmann (1903, 1904), who employed a strain isolated from a turtle, have received the most attention. This and other ' cold-blooded ' strains have been widely examined in the laboratory and in clinical use, and the most reliable investigations indicate that they are devoid of any significant immunizing power (German Commission, 1923).

Immunization by virulent bacilli.

The work of Courmont and Lesieur, Hamberger, Römer and Joseph, Webb, Williams and Barber (1909) and Webb and Williams (1911^1), Bruyant (1911), and others, showed that, even with virulent bacilli, a disease of chronic and slowly progressive type could be produced in the guinea-pig if the infecting dose were reduced to minimal proportions. Such infection was accompanied by a marked resistance to reinfection by small doses, but the immunity did not reach sufficient grade to eliminate the primary infection, and the animals succumbed finally to generalized disease.

Calmette and Guérin (1907, 1908) had already showed that cattle could be infected by the digestive tract with virulent bacilli, without the production of progressive disease ; 50 mgm. of bovine bacilli introduced by the oesophageal sound, in calves, led to limited lymph node lesions, from which the animals apparently recovered. They showed a marked resistance to reinfection for at least 8 months, and after having resisted a massive second dose by the same route, tolerated an intravenous injection of virulent bacilli without evident disease for 6 to 8 months, after which, however, lesions might develop. The important factor in this method appears to have been the same as in the work with guinea-pigs, that the actual infecting dose was very small.

A number of investigators have inoculated themselves, or tuberculous patients, with living bacilli (see Baldwin and Gardner, 1921), with no ill effects, and with the production of only small local lesions, but this represented reinfection in probably every case. Webb and Williams (1911^3) inoculated two infants with repeated doses of living bacilli, starting at one bacillus, and neither had developed tuberculin sensitivity at four months. Webb and Gilbert (1914) inoculated a third infant with doses increasing from 2 to 150 bacilli, and the child developed some small local nodules and lesions of regional lymph nodes, all of which were excised. The child reacted to tuberculin and continued to do so after the excision.

Selter (1921^2, 1922^2, 1923) has more recently employed a preparation which he calls ' vital tuberculin ', made by exhaustive grinding of living cultures in saline, until most of the bacilli are disintegrated, a result which can be obtained only with certain strains and with old cultures. The preparation gave limited lesions in guinea-pigs and produced definite immunity, but the primary strain, in these experiments, was of low virulence. In man, the material was used chiefly for treatment, but

Selter (1925) has inoculated nine infants with emulsions of living bacilli, partially disintegrated by grinding, in doses of 12 to 10,000 bacilli. The injections were made subcutaneously, and one dose of 10,000 and one of 1,000, produced local lesions which broke down and were excised. In two cases a dose of 1,000 gave nodules and lymph node enlargement which disappeared spontaneously. Smaller doses gave very small local lesions with no node involvement. There was no extension of disease, but the children reacted to tuberculin at from 4 weeks to 3½ months. Those receiving the larger doses still reacted strongly at 15 months.

There can be no doubt that in man, in cattle, or in other species with a capacity for developing effective resistance, a small dose of virulent bacilli may be tolerated without producing progressive disease, and the clinical evidence in man amply supports this view, but, as Calmette (1928[1]) points out, the method is too dangerous for practical use. In man, there is the risk both that a certain proportion of cases may fail to limit the infection, and, in all cases, that the lesions may become active at a later date. In cattle there is the danger, already noted, of excretion of virulent bacilli in the milk.

Immunization by avirulent bacilli.

The extent to which the tubercle bacillus may lose its virulence during artificial cultivation has led to many hopes of securing a completely innocuous strain, which would yet produce effective immunity. Many attenuated strains have been used in experimental work, but nearly all have shown some degree of virulence for the guinea-pig. Though the lesions may remain localized for months, the animal finally succumbs with generalized disease. The same is true for bacilli which have been 'weakened' by physical means, such as heat or radiation, or by chemical agents, such as phenol, formaldehyde, halogen compounds, &c. The effect of such treatment is probably in part merely a reduction of the infecting dose, but, in any case, the injected bacilli which remain viable, or their descendants, usually regain sufficient virulence to kill the guinea-pig. That such attenuated or weakened cultures may be apparently harmless to cattle, and yet produce considerable immunity, has been shown in several investigations, but the risk of their retaining or regaining infectivity for man has, in general, precluded their practical use. In this regard, Borrel, Boez and de Coulon (1923) have found that some apparently quite avirulent strains may regain normal virulence after a few passages in the guinea-pig.

In a few cultures the loss of virulence appears to be more complete and permanent. The human strain, R 1, isolated by Trudeau in 1890, and which rapidly lost its virulence (Trudeau, 1897), has been extensively used experimentally by Krause and his associates. According to Krause (1919), it produces in the guinea-pig local lymph node lesions which gradually decrease, but do not disappear, and only occasionally leads to visceral lesions. In his hands, the strain has never given progressive disease, but it has occasionally done so with others (Baldwin and Gardner, 1921; Webb, Ryder and Boissevain, 1925). Guinea-pigs infected with R 1 remain healthy up to two years or more (Krause, 1916; Willis, 1928), and their tuberculin sensitivity becomes very weak, but Krause (1919) found that the bacilli remained alive in the regional and tracheo-bronchial nodes. Resistance to reinfection is comparable to that seen in guinea-pigs with chronic infection by other strains, and Willis (1928) found that with reinfection two years after primary R 1 infection, there was a much less rapid development of lesions than in controls.

Among other strains of this class, may be mentioned the K 1 strain isolated by Koch over forty years ago, the equine strain, possibly of avian type, isolated by Nocard, and used by Vallée (1924), and a strain, BB, isolated by E. Burnet in 1910, and described by Borrel, Boez and de Coulon.

In recent years interest in the problem of preventive inoculation has centred chiefly in the avirulent strain, B.C.G. (Bacille Calmette Guérin), which has been the subject of numerous publications by Calmette and Guérin, and their associates, during the past twenty years. The following summary of their results is based on Calmette's recent reviews (1927, 1928[1 & 2]).

The culture, of bovine origin, was cultivated continuously from 1906 on potato saturated with ox-bile and 5 per cent. glycerin. After four years it had lost virulence for cattle and guinea-pigs, and after 230 transfers on the bile media it was completely avirulent for all domestic animals. The strain has since been maintained on ordinary media, with a transfer to bile media after each 10 subcultures.

Injected in mammals, the culture produces lesions analogous to those given by dead bacilli. There is formation of tubercle tissue, and, after large doses, of cold abscesses, but all the lesions gradually diminish and disappear completely. Reinoculation from lesions has never given active disease, nor has it been possible to restore any degree of virulence by animal passage. In guinea-pigs, subcutaneous doses up to 1·0 mgm. (moist) do not produce progressive disease. Large doses by any route, and particularly intracardiac, cause visceral tubercles, which later disappear. Similar results were obtained in rabbits. Cattle were injected intravenously with doses up to 200 mgm., with no ill-effect, and chimpanzees and monkeys tolerated the culture by injection or by ingestion.

Inoculation of the culture is followed by an immunity which is evident, though incomplete, in guinea-pigs, but of high grade in cattle. Treated guinea-pigs do not resist subcutaneous reinfection with virulent strains, but after conjunctival instillation of such bacilli, which produces progressive disease in normals (Calmette, Guérin and Grysez, 1913), lesions are limited to the cervical lymph nodes for 3 to 5 months, after which the disease becomes generalized. Similarly, treated rabbits resist virulent reinfection for about 6 months, but finally a fatal disease results.

Cattle were inoculated with B.C.G. intravenously (1 to 5 mgm.) or subcutaneously (up to 100 mgm.), and reinjected intravenously with virulent bacilli at periods of 1 to 18 months later. With one exception (18 months), they remained free from lesions, though, in most cases, their abdominal lymph nodes remained infective for guinea-pigs. In an experiment carried out in West Africa, 3 chimpanzees and 16 monkeys were treated by subcutaneous injection of 50 mgm. B.C.G., or by 5 ingestions of 50 mgm. each, and were housed with infected animals, and with normal controls. All the treated animals remained free from tuberculosis, the observation lasting up to 3 years, and those dying of intercurrent disease showed no tuberculous lesions, while the controls, in most cases, succumbed rapidly.

The method was put into practice in cattle in 1921, and in one infected establishment 110 calves were inoculated during 5 years, each animal being reinoculated yearly. Though many were from tuberculous mothers, and were fed on infected milk, all remained healthy, and 30 which were slaughtered were free from lesions.

In 1921, inoculations were begun in new-born infants of tuberculous mothers, the culture being given by ingestion, in 3 doses of 2 mgm. each, during the first two weeks of life. This dose proving innocuous in 120 infants, it was increased to 10 mgm. for each ingestion, which is still the standard amount. Up to 1925, 300 further infants were treated, 67 of them from tuberculous mothers, and the only death from tuberculosis was in an infant of the latter class. In 1924, the method was put into general practice in France, and later in several other countries. According to the latest review (Calmette, 1928[2]), there had been treated, up to October, 1928, 110,000 infants in France and in French colonies.

The object of the treatment is not to produce a permanent immunity, but to give sufficient resistance to carry the child through the period of life at which the disease is most dangerous, and to enable it to go through the usual course of natural immunization by repeated small infections, without the risk of such infections becoming progressive. The experiments in cattle showed that immunity was weakening at a year to eighteen months, and it is desirable that the inoculation should be repeated at yearly intervals.

Independent experimental examination of the culture is still at an early stage, and the results, on the two main questions of lack of virulence, and of production of effective immunity, cannot yet be accepted either as complete substantiation or controversion of Calmette's claims, as set forth in the above summary.

There is general agreement that the virulence is at least of a very low order for man and cattle, with somewhat less agreement as to the guinea-pig. On this point, perhaps the most interesting results, in their general and practical bearing, are those of Petroff (1927[1]), who has succeeded in separating 'rough' and 'smooth' variants of the tubercle bacillus, and who finds (Petroff, Branch and Steenken, 1927) that the 'smooth' colonies of B.C.G. may produce, in the guinea-pig, a fatal and transferable tuberculosis. This finding does not necessarily exclude the use of the culture in man, but it suggests that its harmlessness may depend on certain constant conditions of preparation, and indicates, at least, the need for caution, and the necessity for strict control of the material used for treatment.

As regards the immunity produced under experimental conditions, there is much less agreement, and less complete substantiation of Calmette's results. Some satisfactory results have been produced in cattle, but it is still too early for a critical review.

On the clinical side, the bulk of the existent data are those produced by Calmette and his associates, and space does not permit of the extensive statistical treatment which is necessary for any fair presentation of them. Calmette, however, believes that the tuberculosis incidence in treated children is much lower than in those of the general population, and that the method has already lowered the general death-rate in certain districts.

Immunization by dead bacilli.

In the earlier experiments, numerous workers attempted to produce immunity by treatment with killed bacilli, which, it had been shown, produced anatomical lesions similar to the tubercle of active infection. Many of the experiments showed evidence of a relative resistance to reinfection in the treated animals. This was, however, generally of less degree than the immunity of active infection, to which is possibly due the general belief that the method promises no effective results in practice. Thus, Calmette, Guérin and Breton (1907), Römer (1909[2]), consider the immunizing effect as negligible, though Calmette and Guérin (1914) had more promising results in cattle. Within recent years the '*diaplyte vaccine*' of Dreyer (1923) gave some promising initial results, but on more extensive trial was found ineffective, at least in the guinea-pig.

Raw (1925) has inoculated a large number of children with killed bacilli, and thinks the results, as also those in animals, indicate definite immunization. Langer (1925) obtained evidence of resistance in guinea-pigs treated with heat-killed bacilli, and has treated a number of children, the majority of whom developed cutaneous sensitivity.

In regard to the general disbelief in immunity from dead bacilli, it must be noted that the production of allergy by the same means was for years widely contested, though now clearly established, while, if a relation between the two processes is accepted, it is to be expected that some resistance will result from treatment which leads to a high degree of allergy. Petroff (1927[2]) has recently emphasized this view, and Petroff and Stewart (1926), in a series of over 100 guinea-pigs, sensitized by heat-killed bacilli and reinfected subcutaneously or by intraocular injection, found the average length of life in all treated series greater than in controls —in some series nearly twice as great—while there was striking difference in the extent of the lesions. Their results are actually as outspoken as in many of the classic experiments dealing with active infection, and the immunity conferred on guinea-pigs was at least as great as that described for B.C.G., although the reinfecting doses were large—0·002 to 0·004 mgm. of virulent bacilli. It seems highly probable that results comparable with those claimed for B.C.G. in infants might be obtained by general application of this method on the same plan.

Passive transfer of immunity.

Experimental attempts to transfer immunity to tuberculous infection from immune to normal animals have given, with a very small number of isolated exceptions, unequivocally negative results. Those results which suggest some degree of success are reviewed by Neufeld and Lange (1923).

Baumgarten, Hegler and Kappis (1906), in 4 of 5 calves treated with serum from immunized cattle, obtained a considerable degree of resistance to bovine inoculation, but Baumgarten and Dibbelt (1908) on repeating the experiments on a larger scale, with stricter control, had entirely negative results. Römer (1914), in two sheep treated with serum from immunized sheep, obtained marked resistance to intravenous inoculation of 0·2 mgm. bovine bacilli per 10 kgm. body weight. Two other sheep treated with serum from the same animals, but taken seven months later, showed no resistance. Neufeld and Lange, in similar experiments, had very indefinite results, but thought that some of them might indicate a degree of resistance.

The experimental findings are in harmony with the clinical results obtained from the many sera which have been prepared for therapeutic use. Many of these have been enthusiastically received and much initial success has been claimed for them, but one by one they have dropped into disuse, save, at most, in restricted circles. When the results are reviewed in proper perspective, it seems doubtful whether any of them have produced any results which represent unequivocal evidence of specific immunizing effect.

The Mechanism of Immunity : Allergy and Immunity.

The mechanism of immunity in tuberculosis is, as yet, a matter rather for speculation than for argument. Of the possible factors concerned, allergy has received and still receives the major share of most discussion. This is no doubt due, in part, to the accumulation of a considerable mass of data concerning the allergic condition, for which some degree of precision may be claimed, and to the almost complete lack of such data for other factors ; a state of affairs which may be regarded, according to individual opinion, either as significant or as the result of a chance trend of experimental work. General opinion still seems to regard allergy as a most important factor in resistance to infection, though the view is usually expressed in more tentative form than previously, and is openly challenged in some quarters.

Briefly, the case for allergy rests on the high degree of parallelism, in experimental tuberculosis, between sensitivity and resistance to reinfection, and on the view that on the allergic reaction, as we know it, it is possible to base at least some explanation of such resistance. Opposing opinion claims that the two conditions do not necessarily co-exist, and that, as Calmette (1928[1]) holds, they are independent and their apparent association fortuitous.

The parallelism was recognized and considered significant by most of the experimenters who demonstrated the existence of acquired immunity in tuberculosis, including some, such as Calmette, who now consider the conditions independent. Progressive tuberculous infection appears to be accompanied invariably, at some stage of its course, by allergic sensitivity, and allergy may, indeed, be said to be as characteristic a feature of the disease as is the anatomical tubercle. The allergic phase corresponds, in general, to that during which resistance to reinfection is most evident. In experimental infection, the incubation period required for development is of the same order in the two cases. In the final stages of progressive disease both conditions disappear, and again at about the same period. This parallelism, both in development and in disappearance, is well illustrated in the experiments of Rolland (1914) with intracutaneous reinfection, in which the inoculation served as a test both of allergy and of immunity. Where the intense necrotic reaction was produced, the reinfection was resisted or limited. In early or late stages of the primary disease, where the allergic reaction was weak or lacking, a progressive abscess resulted. It is difficult to regard the association of the necrotic allergic reaction with the failure of reinfection, as observed by Koch and many subsequent workers, as entirely fortuitous, though it must be admitted that it represents a special case. In two further characteristics, of more precise nature than those outlined above, the parallelism may be extended. Passive transfer has almost universally failed, both for allergy and for immunity, and both conditions appear to depend on the action or co-operation of living cells ; and both require for their maintenance a continuance of the antigenic stimulus, and diminish or disappear with recovery and elimination of the infecting agent.

The view that the allergic reaction can, to some extent, explain resistance to reinfection rests on evidence which is far from conclusive, but which is too suggestive to be ignored. In the first place, a considerable number of workers have claimed to demonstrate, during the course of the acute allergic reaction, a lytic process leading to destruction of tubercle bacilli, though this process cannot be said to be finally established, and does not go unchallenged. Secondly, there is very clear evidence that a second infection in the tuberculous animal is influenced by factors which limit the extent, and particularly the rate, of its spread, as compared to a primary infection. There is much to suggest that these factors depend on the allergic condition, but here again the evidence is still inconclusive.

The lytic process has been studied chiefly in the case of tubercle bacilli injected into the peritoneum of the tuberculous guinea-pig. Kraus and Hofer (1912[1 & 2]), Manwaring and Bronfenbrenner (1913), Rist, Léon-Kindberg and Rolland (1914[1]), and others, have described a rapid disappearance of bacilli from the exudate which follows such an injection, and it has been claimed that bacilli can be observed in various stages of disintegration. The disappearance of bacilli from the exudate is in part due to their adhesion to the peritoneal surfaces, but Manwaring and Bronfenbrenner, by scrubbing these surfaces, were able to recover only 55 per cent. of bacilli injected in the tuberculous animal, as against 100 per cent. in the normal. Rist, Léon-Kindberg and Rolland (1914[2]) found the exudates from 7 of 12 guinea-pigs reinoculated intraperitoneally to be non-infective for normals. This result requires confirmation, but most writers have found that peritoneal tubercles developed scantily or not at all, following the second inoculation. Baatz (1920), working in Pfeiffer's laboratory, was unable to obtain any evidence of intraperitoneal lysis, or any clear difference between tuberculous and normal guinea-pigs, as regards the number of bacilli in the exudate. Rist and Rolland (1914) described an extensive disappearance of bacilli inoculated intracutaneously in the tuberculous guinea-pig, during the course of the allergic reaction, a result which the writer, in a small series of animals, has not been able to confirm.

It is particularly to be noted that the evidence most suggestive of an active destruction of tubercle bacilli has been obtained only where the allergic reaction was of high intensity, i.e. where the reinoculation dose was larger than is usual in natural infection.

The tendency to localization of a second infection has been recognized for most animal species which are capable of developing any considerable degree of immunity. Even in the guinea-pig, where immunity is seldom sufficient to prevent an ultimate generalization from a virulent reinoculation, the spread of the new infection is much slower than that of a primary infection by the same organism. The precise work of Krause, and of Willis, has demonstrated that in the normal guinea-pig, intra- or subcutaneous inoculation is followed by a rapid spread of bacilli to the regional lymph nodes, and thence by the main lymphatics to the bloodstream—a process of great importance for the subsequent development of visceral lesions. With intracutaneous inoculation, Willis (1925[1]) found that excision of the inoculation site prevented general infection only when performed within 3 hours, while the regional lymph nodes were infective for normals at 24 hours. Krause (1920) found the internal viscera always infective at 4 days. In guinea-pigs previously immunized with the avirulent strain R 1, Willis (1925[2]) found, after virulent intracutaneous inoculation, that excision of the site prevented general infection up to the seventh day, while the regional lymph nodes were not infective until the third week. General dissemination did not occur before 3 to 4 weeks (Krause and Willis, 1925).

When we consider the mechanism by which dissemination is effected—generally accepted as depending on transport of phagocyted bacilli within mobile cells—it seems clear that the delayed spread must be ascribed to immobilization of such cells, as the result either of mechanical interference, or of injury to the cell. Either event it appears possible to ascribe to the allergic process. It is highly probable that cells carrying tubercle bacilli are constantly exposed, in their passage through the lymphatic tissue of the allergic animal, to some degree of local inflammatory reaction, by which they may be held up. It is also probable that cells of the allergic animal may themselves suffer injury when they take up tubercle bacilli, through an ensuing allergic reaction, extra- or intracellular. Stewart, Long and Bradley (1926), by the supravital staining method, found that the leucocytes of the allergic animal rapidly succumbed on contact with tuberculin *in vivo*, indicating that the leucocytes themselves may become sensitive, and hence susceptible to injury on contact with tubercle bacilli.

The allergic condition thus seems to offer, in itself, a sufficient explanation of the delayed spread of a second infection, and it is to be noted that the same factors will come into play when bacilli are liberated from tuberculous lesions, either of a primary or of a secondary infection. The actual rate of spread is probably about the same in the two cases, the rapid visceral involvement after primary inoculation by injection being largely the result of the initial seeding already described, a point further considered in discussion. For a more extensive presentation of the importance of the allergic inflammatory reaction in delaying the spread of infection in the immune animal, the reader is referred to numerous papers by Krause during the past ten years.

Those who oppose the view that allergy is an important factor in immunity base their case mainly on the claim that either condition may exist without the other. Calmette and his associates, perhaps the most outspoken of this group, offer, in support of the claim, two experimental results (Calmette, 1928[1]). Guinea-pigs became allergic at 8 to 10 days after inoculation with B.C.G., but were not immune to virulent reinfection until the thirtieth day, indicating allergy without immunity. On the other hand, in cattle immunized with B.C.G., the immunity remained demonstrable for several months after the skin reaction failed, that is, immunity without allergy.

The first of these results is, at best, inconclusive, and may well be explained on the ground that immunity is much less readily demonstrable, experimentally, than is allergy, and, in the guinea-pig particularly, is only recognizable when of maximum degree. Evidence has already been presented which indicates that allergy, as judged by the skin reaction, increases progressively from the time it first appears, to reach a maximum at the third to fifth week—the period at which Calmette found immunity to appear.

The second claim, that immunity outlasts allergy is, at first sight, more important, and is the argument most commonly advanced against the relation of the two processes. It must be considered, however, in the light of the capacity of an animal which has once been allergic to redevelop sensitivity at a rate much more rapid than that seen in the normal—a process which has analogies in the field of antibody production.

Willis (1928) found that guinea-pigs, at about 2 years after inoculation with R 1, no longer gave a skin reaction to strong tuberculin, but on virulent reinoculation allergy redeveloped so rapidly that reactions of full intensity were obtained 4 days later. Baldwin and Gardner (1921) obtained similar results in guinea-pigs, and note the importance of latent allergic power. It is obvious that the influence of allergy is not excluded by a negative skin reaction to tuberculin previous to reinoculation; since with the rapid reactivation of sensitivity the reinfecting bacilli may come under the full influence of the allergic reaction with only a limited delay.

Apart from tuberculosis, the relation of allergy to immunity receives much support from the study of microbic infection in general, and particularly of those infections in which spontaneous recovery is the rule. This view was extensively developed by v. Pirquet (1911), and there need only be mentioned here, on the one hand, the febrile reactions of the infectious fevers, and, on the other, the inflammatory processes at the site of localized infections, of which the former probably, and the latter often obviously, represent an increased capacity of the host to react to the specific infecting agent. In both cases there is a tendency for the reaction to reach a maximum intensity immediately prior to the onset of clinical recovery. There is much to suggest, indeed, that bacterial inflammation in general, even that caused by the ordinary ' pyogenic ' organisms, is mainly allergic in nature, that is, dependent on a specific sensitization of the host tissues, leading to a greater reacting capacity towards the specific organism than is possessed by normal unsensitized tissues.

But while, in the writer's view, experimental data strongly suggest that allergy plays an important part in immunity, and while no results so far produced appear to exclude this relation, it cannot be claimed that it offers a full explanation, or even that a high degree of skin sensitivity, in itself, indicates an effective immunity. According to recent investigations* the South African natives develop a very high degree of allergy as judged by the intracutaneous reaction, yet they have little capacity for limiting a progressive infection, and succumb with extensive lesions of the type seen in children or in guinea-pigs. The same discrepancy may be observed in individuals of the more resistant races, and, while the cutaneous tuberculin reaction may not tell the whole story of the allergic condition, it still seems necessary to assume that other factors than allergy must be involved in the complex process of immunity.

As to the nature of such factors, indications are extremely vague. Among the more definite possibilities to be considered are processes which, without leading to gross tissue reactions, or to active destruction of infecting bacilli, may yet inhibit the growth of the latter, so that a condition of latent infection is produced. Calmette and Guérin (1908) found that a virulent inoculation in immunized cattle might produce no appreciable lesions for months, but that the lymph nodes of these animals continued to be infective for guinea-pigs, and that when, after a year or two, the original immunity had disappeared, progressive tuberculous disease might arise. Similarly, in rabbits immunized with B.C.G., a virulent infection may be held in check for several months, but finally produce generalized disease (Calmette, 1927, 1928[1]). Many analogous results have been described, and the whole question of latency of infection is one of great importance, both theoretical and practical, in the clinical study of tuberculosis.

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* Personal communication from Professor S. L. Cummins.

As to whether an alteration in virulence of infecting bacilli, as a result of life within the animal body, may occur, or may have a relation to immunity, there is little definite information. There are not many recorded results which indicate a definite attenuation of tubercle bacilli in hosts of susceptible species, and there are, on the other hand, many results showing retention of virulence, even in highly immune hosts. The dissociation of tubercle bacilli into ' rough ' and ' smooth ' variants, of differing virulence, by Petroff (1927[1]) suggests a wide field for investigation in this connection.

A process whose significance it is difficult to assess is the increased rate at which the immune animal, as compared with the normal, develops tubercle tissue in response to a new infection. This was particularly evident in the experiments of Debré and Bonnet (1922) and of Krause and Peters (1920) already described, in which intracutaneous reinoculation in the immune animal produced rapidly developing but finally abortive tuberculous lesions. If, according to the view favoured by present opinion, tubercle tissue is largely a reaction to the fatty constituents of the bacillus, it is difficult to consider the accelerated lesion formation as a product of allergy, in the sense in which the latter has been considered, i.e. a specific sensitivity to bacillary protein, though it would still come within the scope of v. Pirquet's original conception as an increased capacity to react. Boquet and Nègre (1926) consider the accelerated cellular reaction in tuberculosis as identical with v. Pirquet's accelerated vaccinal reaction, and with analogous processes in leprosy, Leishmaniasis, and epizootic lymphangitis of solipeds. They think the process independent of allergy, but an important factor in immunity, the latter view being also expressed by Krause and Peters.

Discussion.

From both theoretical and practical standpoints the most particular problem involved in the immunity of tuberculosis is its relatively low effectiveness, as compared with that seen, for example, in the acute infectious fevers. In a disease such as smallpox the normal course, barring an acutely fatal result, is complete, and relatively rapid, spontaneous recovery, with elimination of the infecting agent, and with a resistance to reinfection which remains effective for years after the stimulus of the specific antigen has ceased. In tuberculosis the disease, in man, follows no constant or characteristic course, but may show, in different individuals of the same community, complete clinical recovery, more or less permanent latency of infection, intermittently or steadily progressive disease of varying chronicity, or a rapidly progressive and fatal process.

There can be little doubt that these variations represent, to a large extent, variations in the effectiveness of a specific acquired immunity, though many non-specific factors must be involved. Despite the clinical variations, epidemiological data clearly indicate that immunity plays a real and important part in the disease, especially when the general course of disease in civilized adults is compared with that in children, or in races only recently exposed to infection. Experimentally, the existence of immunity is clearly demonstrable, but much more readily as a resistance to reinfection during the course of the primary disease than as a complete and permanent protection.

But this immunity produces no such dramatic results as are seen in the acute infectious fevers. Experimental reinoculation of the tuberculous animal brings out the paradox that the animal which can resist a new and virulent infection is still unable to prevent the progress of the primary infection. Even where recovery takes place, elimination of the infecting agent is, at best, a very slow process, and it is still questionable whether, in man, it is ever complete. Again, the resistance to reinfection tends to fall as the antigenic stimulus diminishes with recovery, and experimental results suggest that it finally disappears within a year or two, at most, after complete elimination of the primary infection. In some experimental results, the immunity seems to bear a direct relation to the extent, up to a point, of the primary disease, but in man this is more difficult to demonstrate, and the relation often appears to be an inverse one.

A characteristic feature of the immunity of tuberculosis is that it is a relative immunity only, its effectiveness varying with the reinfection dosage. In the guinea-pig, a complete immunity to virulent infection has been observed only with the most minute doses possible, while in all species there appears to be a limit of dosage, beyond which progressive disease will result, even with maximum immunity. While the same is probably true in principle, for all infections, even for those in which the immunity, under natural conditions of exposure, appears to be absolute, it is a point of particular importance in tuberculosis, for two reasons. The reinfection dosage which the member of a civilized community is called upon to resist, and particularly when in contact with active cases, is probably much larger, relative to the degree of immunity developed, than is the case with most of the acute infections. And, as a result of the slowness with which even the immune host finally destroys and eliminates tubercle bacilli which have gained a foothold, repeated small infections may have a cumulative effect, so that resistance is finally overcome rather than progressively increased. The relation between the degree of immunity and the size of infection it is called upon to resist seems to be the essential factor in deciding not only the success or failure of a new infection, but the course of an established infection, whether primary or secondary, and it is to variation in this relation, rather than in the absolute degree of immunity, that the varying clinical types noted should be ascribed.

An explanation of this complex problem must wait upon deeper knowledge of the processes of immunity. We cannot, at present, even decide whether the forces of specific immunity in tuberculosis are quantitatively weaker, or qualitatively different from those in the acute infections, in which they are so much more effective. It is frequently assumed that the tubercle bacillus is particularly resistant to the processes of immunity, a conception suggested by its waxy structure, and its impermeability to dyes. This view receives some support from the slowness with which the bacilli are destroyed within the body. Even heat-killed bacilli may remain intact and acid-fast in the peritoneum of the guinea-pig for at least a year or more. Neufeld (1921), however, points out that similar conditions of immunity exist in infections such as malaria, where the infecting agent is of delicate nature. Again, the persistence of the primary infection, despite resistance to a new inoculation, has suggested the selective development of resistant types of bacilli, during the course of disease. Römer and Joseph (1910[1]) had apparently disposed of this idea when they showed that tuberculous guinea-pigs were resistant to reinoculation with material from their own lesions. More recently, however, Paraf (1925[1]) has found that while this held for small dosage, tuberculous guinea-pigs appeared to be less resistant to large doses of pus from their own lesions than to similar doses from other animals, even those inoculated with the same strain of bacillus—a result which would require extensive confirmation.

While it is unsafe to assume that the essential processes of immunity differ in different infections, it is probable that multiple factors are involved, and that these may vary in their relative importance. And there is much to suggest that the immunity of tuberculosis is qualitatively different, if only in this sense, from that of the acute infections. Two features which point in this direction are the failure of passive transfer, indicating that circulating antibodies do not, in themselves, give effective protection, and the impermanence of the acquired immunity, both of which, as already noted, apply equally to allergy. Immunity in tuberculosis, like allergy, appears to be a function of living cells, or, at least, to require their co-operation. Whether only such cells as the mobile phagocytes are involved, or whether all the tissues are active, is not indicated as yet. It appears certain that phagocytosis, by itself, does not insure the death of the tubercle bacillus.

But, with all these considerations, it must be recalled that when tubercle bacilli are inoculated, in suitable dose, into the undiseased tissues of the tuberculous animal, the host shows sufficient immunity, and the bacilli sufficient susceptibility, to allow of inhibition, or apparent elimination of the second infection. This brings us to the critical difference in situation between these bacilli and those of the primary disease, that the former are exposed directly to the action of healthy, vascularized tissue, with its acquired capacity for resistance, while the latter are embedded in the avascular tissue, or necrotic material, of the tuberculous lesion. In this situation the enclosed bacilli must receive a considerable degree of protection, both from mobile phagocytes, and from whatever activity is to be ascribed to serum-borne antibodies. The conception is in harmony not only with pathological findings, such as the long persistence of viable bacilli, even within healing lesions, but also with many experimental results which suggest that, in resistance to reinfection, the main action takes place before the protective wall of tubercle tissue is formed. This is obviously so where resistance is complete. It is also probably the case, to a large extent, when, as usual in the guinea-pig, immunity is only sufficient to alter the disease resulting from reinfection in the direction of chronicity and slowness of spread. The work of Krause, and of Willis, already reviewed, suggests that, in primary infection in the guinea-pig, a large proportion of the visceral lesions owe their origin to an initial seeding of the general circulation during the first few days. In the immune animal, however, this initial passage of injected bacilli through the lymphatic system to the blood-stream was almost entirely inhibited, the effect of which would be that visceral lesions would appear much more slowly and more scantily than in the normal. Thus, what appears to be a continued effect of a primary immunity may well owe its nature to an action which has taken place before the second infection has given rise to tubercle formation.

The degree of protection afforded by the tubercle tissue must vary greatly with the nature of the lesion, and with the situation of individual bacilli. It is probable that it is never complete for all the bacilli in a lesion, but that it is more or less so, at any one time, for a certain proportion of them. With changing local conditions, all the bacilli in a lesion might come, in their turn, under the influence of a high-grade immunity. That such an immunity may be effective against bacilli within well-developed lesions is indicated by clinical and pathological data, and by such experimental results as those of Krause and Peters (1920) in which intracutaneous inoculation, in immune guinea-pigs, produced tuberculous lesions which went on to ulceration, but which finally healed and disappeared. The protection must, then, be regarded as relative at best, but this term is applicable to most of the processes involved in tuberculosis, without excluding their importance. As determining the character of the disease, the protection factor appears, in the writer's opinion, as the most important of those which can be clearly envisaged at the present time.

There remains, however, one important point which the theory of protection of bacilli within the lesion does not cover—the progressive formation of new tubercles as a result of liberation of bacilli from older lesions. Such bacilli are obviously unprotected for a time, yet the new tubercles continue to arise, even where there appears to be a considerable grade of immunity. The most satisfactory explanation seems to be that emphasized by Neufeld (1921), that immunity in tuberculosis is characteristically vacillating, and the spread of disease correspondingly intermittent, which accords with the frequent pathological picture of the development of lesions in successive crops. The explanation is, at best, superficial, and brings us back to the fundamental problem of the nature of immunity, which still awaits solution.

It is to be noted that the walling-off of bacilli will also hinder the egress of bacterial constituents, and the absence of allergic reaction about mature lesions has already been noted as indicating the slow rate at which these substances leak out. When we consider, however, the degree of immunity which may result from a small lesion, and the general failure of resistance with advanced disease, it seems doubtful if a reduction of the antigenic stimulus from this cause is of great importance. If a failure of stimulus is responsible for the relatively low immunity of tuberculosis, it is probably a qualitative rather than a quantitative failure. Neufeld considers that in tuberculosis, as in other infections where the immunity is of similar type, the deciding factor is the poor antigenic quality of the infecting agent.

Among such analogous diseases, Neufeld includes syphilis, trypanosomiasis, malaria and Texas fever of cattle, while others might be added. In all these infections we may have, as in tuberculosis, the condition of resistance to reinfection, combined with inability to eliminate the primary infection. The clearest analogy is, perhaps, with syphilis, in which this state of affairs is even more evident, clinically, than in tuberculosis, and in which it has also become obvious, with modern treatment, that the immunity may not long outlast complete recovery. A feature common to all these infections is that the type of immunity which develops with natural infection gives little promise that any striking therapeutic results, or any permanent prophylactic effects, are to be obtained from artificial immunization.

This consideration, in tuberculosis, must dominate the whole problem of specific treatment. To quote Neufeld, ' He who seeks, in tuberculosis, the production of an immunity similar to that obtained for smallpox, seeks that which is not '. From the therapeutic side, the results of treatment by tuberculin, or by tubercle bacillus vaccines, are very difficult to assess. While a large body of clinicians firmly believe that beneficial results are obtained, there is no doubt that overdosage may lead to definitely harmful effects. Favourable results appear to depend on the production of a low grade of focal reaction, and it is doubtful whether any effective system of treatment is free from the danger that this grade may be exceeded to a harmful extent. The outlook for specific immunization as a preventive measure is much brighter, even though the immunity may be shortlived. Even a low-grade immunity is sufficient to enable the average human to resist a new infection of small size, while the exposure to such infections which life in a civilized community entails at present, will in most cases ensure the maintenance of the immunity

What is necessary is that the individual shall be given sufficient artificial immunity to resist the earliest natural infections to which he is exposed, and thus to carry him through to the relatively immune state of the average adult. Whether such a result will be obtained by avirulent inoculation, as B.C.G., by the use of killed bacilli, or by other means, remains to be decided, but it seems likely that the near future will see important advances in this direction.

REFERENCES.

ARLOING, F. & DUFOURT, A., 1924, *Rev. Tuberc.*, Paris, 3, **5**, 399.

ARLOING, S., 1903, *J. Physiol. Path. gén.*, **5**, 677.

ARMAND-DELILLE, P., 1923, *Rev. Tuberc.*, Paris, 3 s., **4**, 399.

AUSTRIAN, C. R., 1913, *Johns Hopk. Hosp. Bull.*, **24**, 11, 141.

AUSTRIAN, C. R. & FRIED, H., 1913, *Johns Hopk. Hosp. Bull.*, **24**, 280.

BAATZ, 1920, *Zbl. Bakt.*, Abt. I, Orig., **84**, 81.

BAIL, O., 1904, *Wien. klin. Wschr.*, **17**, 846; 1910, *Z. ImmunForsch.*, **4**, 470.

BALDWIN, E. R., 1910, *J. Med. Res.*, **22**, 189.

BALDWIN, E. R. & GARDNER, L. U., 1921, *Amer. Rev. Tuberc.*, **5**, 429.

BAUMGARTEN, P. & DIBBELT, W., 1908, *Arb. path. Anat. Bakt.*, **6**, 52.

BAUMGARTEN, P. & HEGLER, C., 1906, *Arb. path. Anat. Bakt.*, **5**, 208.

BAUMGARTEN, P. & KAPPIS, A., 1906, *Arb. path. Anat. Bakt.*, **5**, 355.

v. BEHRING, E., 1902, *Beitr. exp. Ther.*, Hefte **5**, **7** & **8**.

BESSAU, G., 1916, *Berl. klin. Wschr.*, **53**, 801.

BEZANÇON, F. & DE SERBONNES, H., 1914, *Ann. Méd.*, **1**, 129.

BLOOMFIELD, A. L. & MATEER, J. G., 1919, *Amer. Rev. Tuberc.*, **3**, 166.

BLUMENBERG, W., 1925, *Beitr. Klin. Tuberk.*, **61**, 509.

BOECKER, E., 1923, *Z. Hyg. InfektKr.*, **101**, 1.

BOECKER, E. & NAKAYAMA, J., 1923, *Z. Hyg. InfektKr.*, **101**, 11.

BOQUET, A. & NÈGRE, L., 1923, *C.R. Soc. Biol.*, Paris, **88**, 1013; 1926, *Ann. Inst. Pasteur*, **40**, 11.

BOQUET, A., NÈGRE, L. & VALTIS, J., 1928, *C.R. Soc. Biol.*, Paris, **99**, 9.

BOROK, M. R. & PICK, M. M., 1926, *Beitr. Klin. Tuberk.*, **63**, 123.

BORREL, A., 1908, *Bull. Soc. Path. exotique*, **1**, 420.

BORREL, A., BOEZ, L. & DE COULON, A., 1923, *Ann. Inst. Pasteur*, **37**, 1012.

BRUYANT, L., 1911, *C.R. Soc. Biol.*, Paris, **71**, 143.

BURNET, E., 1908, *C.R. Soc. Biol.*, Paris, **65**, 307.

CALMETTE, A., 1907, *C.R. Acad. Sci.*, Paris, **144**, 1324; 1927, *La vaccination préventive contre la tuberculose*, Paris (Masson et Cie); 1928^1, *L'infection bacillaire et la tuberculose*, 3rd ed., Paris (Masson et Cie); 1928^2, *Ann. Inst. Pasteur*, **42**, Dec. Suppl.

CALMETTE, A., BRETON, M. & PETIT, G., 1907, *C.R. Soc. Biol.*, Paris, **63**, 296.

CALMETTE, A. & GUÉRIN, C., 1907, *Ann. Inst. Pasteur*, **21**, 525; 1908, *ibid.*, **22**, 689; 1914, *ibid.*, **28**, 329.

CALMETTE, A., GUÉRIN, C. & BRETON, M., 1907, *Ann. Inst. Pasteur*, **21**, 401.

CALMETTE, A., GUÉRIN, C. & GRYSEZ, V., 1913, *C.R. Soc. Biol.*, Paris, **74**, 310.

CASPARI, J., 1925–6, *Z. Tuberk.*, **44**, 447.

CELLA, F. A. DELLA, 1904, *Zbl. Bakt.*, Abt. I, Orig., **36**, 12.

COHN, S., 1907, *Berl. klin. Wschr.*, **44**, 1507.

COULAUD, E., 1921, *Bull. Soc. méd. Hôp. Paris*, **45**, 155; 1923, *C.R. Soc. Biol.*, Paris, **89**, 1023.

COURMONT, J. & LESIEUR, 1908, *C.R. Soc. Biol.*, **64**, 882.

COURMONT, P., 1927, *Rev. Tuberc.*, Paris, 3 s., **8**, 145.

CRAWFORD, A. B., 1923, *J. Amer. Vet. Med. Ass.*, **64**, 228; 1926, *ibid.*, **69**, 579; 1927, *Amer. Rev. Tuberc.*, **15**, 111.

DEBRÉ, R. & BONNET, H., 1922, *C.R. Soc. Biol.*, Paris, **87**, 449.

DEBRÉ, R. & PAPP, K., 1926, *C.R. Soc. Biol.*, Paris, **95**, 29.

DEBRÉ, R. & PARAF, J., 1920, *C.R. Soc. Biol.*, Paris, **83**, 1598.

DEBRÉ, R., PARAF, J. & DAUTREBANDE, L., 1921, *Ann. Méd.*, **9**, 443, 454.

DECRESSAC, G. & JACQUELIN, A., 1924, *C.R. Soc. Biol.*, Paris, **91**, 272.

DETRE-DEUTSCH, L., 1904, *Wien. klin. Wschr.*, **17**, 764.
DIENES, L., 1928, *J. Immunol.*, **15**, 153.
DIENES, L. & SCHOENHEIT, E. W., 1925, *Amer. Rev. Tuberc.*, **11**, 146.
DIENES, L., SCHOENHEIT, E. W. & SCHEFF, L. D., 1925, *Amer. Rev. Tuberc.*, **11**, 151.
DIETRICH, W., 1921, *Deuts. med. Wschr.*, **47**, 406.
DOERR, R., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, Jena, **2**, 1109.
DORSET, M., HENLEY, R. R. & MOSKEY, H. E., 1926, *J. Amer. Vet. Med. Ass.*, **70**, 373.
DREYER, G., 1923, *Brit. J. Exp. Path.*, **4**, 146.
DUNKIN, G. W., 1927, *Vet. Rec.*, **7**, 593.
ENRIGHT, J. J. & RETTGER, L. F., 1924, *Amer. Rev. Tuberc.*, **10**, 78.
EPSTEIN, A., 1891, *Prag. med. Wschr.*, **16**, 4, 13.
EPSTEIN, B., 1921, *Jahrb. Kinderh.*, **95**, 52, 64.
ESCHERICH, 1892, *Jahrb. Kinderhlk.*, 2 n. F., **33**, 369.
FERNBACH, H., 1926, *Beitr. Klin. Tuberk.*, **63**, 60.
FRANCESCHELLI, D., 1914, *Z. ImmunForsch.*, Tl. I, Orig., **20**, 309.
FRIEDMANN, F., 1903, *Deuts. med. Wschr.*, **29**, 953 ; 1904, *ibid.*, **30**, 166, 1673.
v. FRIEDRICH, W., 1921, *Z. Tuberk.*, **35**, 200.
FRISCH, A. V. & KLIMESCH, E., 1924, *Beitr. Klin. Tuberk.*, **58**, 261.
FRISCH, A. V. & SILBERSTERN, E., 1924, *Beitr. Klin. Tuberk.*, **58**, 264.
FÜRTH, 1920, *Z. Hyg. InfektKr.*, **91**, 197.
GABBE, E., 1923, *Biochem. Z.*, **141**, 523.
GAUVAIN, H. J., 1917, *Lancet*, Lond., ii, 519.
GERMAN COMMISSION ON FRIEDMANN METHODS, 1923, *Deuts. med. Wschr.*, **49**, 483.
GRIFFITH, A. S., 1913, *J. Path. Bact.*, **17**, 323.
GRYSEZ, V., 1913, *C.R. Soc. Biol.*, Paris, **75**, 279.
GRYSEZ, V. & PETIT-DUTAILLIS, D., 1912, *C.R. Soc. Biol.*, Paris, **73**, 728.
HAMBURGER, F., 1909, *Beitr. Klin. Tuberk.*, **12**, 259 ; 1910, *ibid.*, **17**, 231.
HAMBURGER, F. & TOYOFUKU, T., 1910, *Beitr. Klin. Tuberk.*, **17**, 237.
HELMHOLZ, H. F., 1909, *Z. ImmunForsch.*, Tl. i, Orig., **3**, 371.
HENLEY, R. R., DORSET, M. & MOSKEY, H. E., 1927, *J. Amer. Vet. Med. Ass.*, **72**, 363.
HOLLO, J. & AMAR, R., 1921, *Beitr. Klin. Tuberk.*, **47**, 357.
HOLST, P. M., 1921, *J. Hyg.*, Camb., **20**, 342 ; 1921–2, *Tubercle*, Lond., **3**, 249.
JOHNSON, T. B. & COGHILL, R. D., 1926, *Trans. Nat. Tuberc. Ass.*, N.Y., 22nd Meeting, 277.
JOHNSON, T. B. & RENFREW, A. G., 1928^1, *Amer. Rev. Tuberc.*, **17**, 508 ; 1928^2, *ibid.*, **18**, 505.
JOSEPH, K., 1910, *Beitr. Klin. Tuberk.*, **17**, 461.
JOUSSET, A., 1921, *Ann. Inst. Pasteur*, **35**, 603.
KLEINSCHMIDT, H., 1918, *Med. Klinik.*, **14**, 1153.
KLEMPERER, F., 1914, *Beitr. Klin. Tuberk.*, **30**, 433.
KLINGMULLER, V., 1902, *Arch. Dermat. Syph.*, Wien, **60**, 109 ; 1903, *Berl. klin. Wschr.*, **40**, 778.
KLOPSTOCK, F., 1924, *Z. ImmunForsch.*, **40**, 27.
KLOSE, E., 1910, *Deuts. med. Wschr.*, **36**, 2239.
KOCH, R., 1890, *Deuts. med. Wschr.*, **16**, 1029 ; 1891^1, *ibid.*, **17**, 101 ; 1891^2, *ibid.*, **17**, 1189 ; 1897, *ibid.*, **23**, 209.
KOCH, R., SCHÜTZ, W., NEUFELD, F. & MIESSNER, H., 1905, *Z. Hyg. InfektKr.*, **51**, 300.
KRAUS, R. & HOFER, G., 1912^1, *Wien. klin. Wschr.*, **25**, 1111 ; 1912^2, *Deuts. med. Wschr.*, **38**, 1227.
KRAUS, R., LÖWENSTEIN, E. & VOLK, R., 1911, *Deuts. med. Wschr.*, **37**, 389.
KRAUSE, A. K., 1911, *J. Med. Res.*, **24**, 361, 469 ; 1916, *ibid.*, **35**, 1 ; 1919, *Amer. Rev. Tuberc.*, **3**, 1 ; 1920, *ibid.*, **4**, 135 ; 1927, *ibid.*, **15**, 137.
KRAUSE, A. K. & PETERS, D., 1920, *Amer. Rev. Tuberc.*, **4**, 551.
KRAUSE, A. K. & WILLIS, H. S., 1925, *Tubercle*, Lond., **6**, 438.
KROMPECHER, E., 1900, *Ann. Inst. Pasteur*, **14**, 723.
KYRLE, 1920, *Arch. Derm. Syph.*, Wien, **125**, 481.
LANGE, B. & FREUND, R., 1926, *Z. Hyg. InfektKr.*, **105**, 571.

Lange, B. & Lange, E., 1922, *Deuts. med. Wschr.*, **48,** 248.

Lange, L. B., 1924, *J. Med. Res.*, **44,** 293.

Langer, H., 1925, *Deuts. med. Wschr.*, **51,** 513.

Lelong, M. & Rivalier, E., 1923, *C.R. Soc. Biol.*, Paris, **88,** 327.

Levy, F., 1908, *Deuts. med. Wschr.*, **34,** 94.

Löwenstein, E., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, Jena, **5,** 660.

Löwenstein, E. & Pick, E. P., 1911, *Biochem. Z.*, **31,** 142.

Löwenstein, E. & Rappoport, E., 1904, *Z. Tuberk.*, **5,** 485.

Long, E. R., 1924, *Amer. Rev. Tuberc.*, **9,** 215 ; 1925, *J. Infect. Dis.*, **37,** 368 ; 1926, *Amer. Rev. Tuberc.*, **13,** 441.

Long, E. R. & Seibert, F. B., 1925, *J. Amer. Med. Ass.*, **85,** 650 ; 1926^1, *Amer. Rev. Tuberc.*, **13,** 398 ; 1926^2, *Trans. Nat. Tuberc. Ass.*, Washington, 22nd Meeting, 270.

Long, E. R. & Seyfarth, M., 1924, *Amer. Rev. Tuberc.*, **9,** 254.

Lustig, A., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, Jena, **2,** 1362.

MacFadyean, J., Sheather, A. L., Edwards, J. T. & Minett, F. C., 1913, *J. Comp. Path.*, **26,** 327.

McJunkin, F. A., 1921, *J. Exp. Med.*, **33,** 751.

Manaud, A., 1909, *C.R. Soc. Biol.*, Paris, **66,** 502.

Mantoux, C., 1908, *C.R. Acad. Sci.*, Paris, **147,** 355.

Mantoux, C. & Perroy, 1911, *C.R. Soc. Biol.*, Paris, **70,** 974.

Manwaring, W. H. & Bronfenbrenner, J., 1913, *J. Exp. Med.*, **18,** 601.

Markert, H. J., 1924, *Z. ImmunForsch.*, **40,** 172.

Medlar, E. M., 1926, *Amer. J. Path.*, **2,** 275, 291.

Mendel, F., 1908, *Med. Klinik.*, **4,** 402.

Meyer, S., 1923, *Z. Hyg. InfektKr.*, **97,** 433.

Moro, E., 1908, *Münch. med. Wschr.*, **55,** 216 ; 1920, *ibid.*, **67,** 1253.

Moussu, G. & Mantoux, C., 1908, *C.R. Acad. Sci.*, Paris, **147,** 502.

Mueller, J. H., 1926, *J. Exp. Med.*, **43,** 1.

Nakayama, J., 1924, *Z. Hyg. InfektKr.*, **102,** 581.

Nègre, L., 1924, *Rev. Tuberc.*, Paris, 3 s., **5,** 161.

Nehring, E., 1923, *Z. Tuberk.*, **38,** 182.

Neufeld, F., 1903, *Deuts. med. Wschr.*, **29,** 653 ; 1921, *Z. Tuberk.*, **34,** 312.

Neufeld, F. & Dold, 1912, *Arb. GesundhAmt.*, Berl., **38,** 275.

Neufeld, F. & Lange, B., 1923, *Z. Hyg. InfektKr.*, **98,** 215.

Nicolle, M., 1908, *Ann. Inst. Pasteur*, **22,** 237.

Nicolle, M. & Abt, G., 1908, *Ann. Inst. Pasteur*, **22,** 132.

Nitschke, W., 1924, *Z. Hyg. InfektKr.*, **102,** 587.

Nobécourt, P. & Paraf, J., 1919, *Bull. Soc. méd. Hôp. Paris*, **43,** 1013 ; 1920, *Pr. méd.*, **28,** 133.

Onaka, M., 1910, *Z. ImmunForsch.*, Tl. I, Orig., **7,** 507.

Opie, E. L., 1924, *J. Immunol.*, **9,** 231.

Ornstein, G. G. & Steinbach, M. M., 1925, *Amer. Rev. Tuberc.*, **10,** 668.

Paraf, J., 1923, *C.R. Soc. Biol.*, Paris, **88,** 1191 ; 1925^1, *ibid.*, **92,** 694 ; 1925^2, *ibid.*, **92,** 792.

Parish, H. J. & Okell, C. C., 1929, *J. Path. Bact.*, **32,** 51.

Petroff, S. A., 1923, *Amer. Rev. Tuberc.*, **7,** 412 ; 1924, *J. Immunol.*, **9,** 309 ; 1927^1, *Proc. Soc. Exp. Biol.*, N.Y., **24,** 632 ; 1927^2, *10th Collection of the Studies of the Edward L. Trudeau Foundation.*

Petroff, S. A., Branch, A. & Steenken, W., Jr., 1927, *Proc. Soc. Exp. Biol.*, N.Y., **25,** 14.

Petroff, S. A. & Stewart, F. W., 1925, *J. Immunol.*, **10,** 677 ; 1926, *J. Immunol.*, **12,** 97.

Philibert, A. & Cordey, F., 1925, *Ann. Méd.*, **17,** 5.

Pickert, M. & Löwenstein, E., 1908, *Deuts. med. Wschr.*, **34,** 2262.

v. Pirquet, C., 1907^1, *Wien. med. Wschr.*, **57,** 1370 ; 1907^2, *Berl. klin. Wschr.*, **44,** 644 ; 1908, *Deuts. med. Wschr.*, **34,** 1297 ; 1911, *Arch. Intern. Med.*, **7,** 383.

Preisich, 1907, *Budapester Aertzverein*, Nov. 9th.

Prudden, T. M. & Hodenpyl, E., 1891, *N.Y. Med. J.*, **53,** 697.

Raw, N., 1925, *Proc. R. Soc. Med.* (*Sect. Compar. Med.*), **18**, 25.

Ray, L. W. & Shipman, J. S., 1923, *Amer. Rev. Tuberc.*, **7**, 88.

Rist, E. & Léon-Kindberg, M., 1914, *Rev. Tuberc.*, Paris, 2 s., **11**, 51.

Rist, E., Léon-Kindberg, M. & Rolland, J., 1914^{1}, *Ann. Méd.*, **1**, 310; 1914^{2}, *ibid.*, **1**, 375.

Rist, E. & Rolland, J., 1914, *Ann. Méd.*, **2**, 13.

Römer, P. H., 1908, *Beitr. Klin. Tuberk.*, **11**, 79; 1909^{1}, *ibid.*, **12**, 185; 1909^{2}, *ibid.*, **13**, 2; 1914, *Deuts. med. Wschr.*, **40**, 533.

Römer, P. H. & Joseph, K., 1909, *Beitr. Klin. Tuberk.*, **14**, 1; 1910^{1}, *ibid.*, **17**, 287; 1910^{2}, *ibid.*, **17**, 427.

Rolland, J., 1914, *Étude sur le phénomène de Koch*, Thèse de Paris (G. Steinheil).

Ruppel, W. G., 1898, *Hoppe-Seyl. Z.*, **26**, 218; 1900, *Beitr. exp. Ther.*, H. **4**, 87.

Sabin, F. R. & Doan, C. A., 1927, *J. Exp. Med.*, **46**, 645.

Scheff, L. D., 1925, *Amer. Rev. Tuberc.*, **12**, 45.

Schittenhelm, A. & Weichardt, W., 1912, *Z. exp. Path. Ther.*, **11**, 69.

Schmidt, R., 1920, *Deuts. Arch. klin. Med.*, **131**, 1.

Schmidt, R. & Kraus, O., 1919, *Med. Klin.*, **15**, 503.

Schonfeld, H., 1923–4, *Mschr. Kinderhlk.*, Orig., **27**, 602.

Seibert, F. B., 1926, *Am. Rev. Tuberc.*, **13**, 431; 1928^{1}, *ibid.*, **17**, 394; 1928^{2}, *ibid.*, **17**, 402.

Seibert, F. B. & Long, E. R., 1926^{1}, *Amer. Rev. Tuberc.*, **13**, 404; 1926^{2}, *ibid.*, **13**, 408.

Seligmann, E. & Klopstock, F., 1921, *Z. ImmunForsch.*, Tl. I, Orig., **33**, 467.

Selter, H., 1920, *Deuts. med. Wschr.*, **46**, 650; 1921^{1}, *Z. ImmunForsch.*, Tl. I, Orig., **32**, 325; 1921^{2}, *Deuts. med. Wschr.*, **47**, 525; 1922^{1}, *ibid.*, **48**, 54; 1922^{2}, *Z. Hyg. InfektKr.*, **95**, 159; 1923, *ibid.*, **98**, 192; 1925, *Deuts. med. Wschr.*, **51**, 1181; 1926, *Trans. Nat. Tuberc. Ass.*, Washington, 22nd Meeting, 276.

Selter, H. & Tancré, 1924–5, *Beitr. Klin. Tuberk.*, **60**, 439.

Slatinéanu, A., 1907, *Revista medicale*, Bucarest, June.

Sons, E. & Mikulicz-Radecki, F. v., 1921, *Deuts. med. Wschr.*, **47**, 735.

Sorgo, J., 1911, *Deuts. med. Wschr.*, **37**, 1015.

Stewart, F. W., 1925, *Amer. J. Path.*, **1**, 495.

Stewart, F. W., Long, P. H. & Bradley, J. I., 1926, *Amer. J. Path.*, **2**, 47.

Stewart, F. W. & Rhoads, C. P., 1926, *Amer. J. Path.*, **2**, 571.

Synwoldt, I., 1920, *Deuts. med. Wschr.*, **46**, 455.

Toenniessen, E., 1922, *Münch. med. Wschr.*, **69**, 957; 1924, *ibid.*, **50**, 629.

Toenniessen, E. & Friedrich, H., 1926, *Deuts. med. Wschr.*, **52**, 1979.

Trudeau, E. L., 1897, *Brit. Med. J.*, ii, 1849.

Trudeau, E. L., Baldwin, E. R. & Kinghorn, H. M., 1904, *J. Med. Res.*, **12**, 169.

Uhlenhuth, P. & Lange, L., 1920, *Deuts. med. Wschr.*, **46**, 1407.

Ungermann, E., 1915, *Arb. GesundhAmt.*, Berl., **48**, 381.

Vallée, H., 1924, *C.R. Acad. Sci.*, Paris, **178**, 152.

Vallée, H. & Rossignol, 1906, *Rev. Tuberc.*, Paris, 2 s., **2**, 217, 466.

Webb, G. B. & Gilbert, G. B., 1914, *J. Amer. Med. Ass.*, **63**, 1098.

Webb, G. B., Ryder, C. T. & Boissevain, C. H., 1925, *Amer. Rev. Tuberc.*, **11**, 247.

Webb, G. B. & Williams, W. W., 1911^{1}, *J. Med. Res.*, **24**, 1; 1911^{2}, *J. Amer. Med. Ass.*, **57**, 1431.

Webb, G. B., Williams, W. W. & Barber, M. A., 1909, *J. Med. Res.*, **20**, 1.

Weber, A. & Titze, C. W., 1907, *TuberkArb.*, Berl., Heft **7**, 1; 1908, *ibid.*, Heft **9**, 1.

Weber, A., Titze, C. W. & Jorn, 1910, *TuberkArb.*, Berl., Heft **10**, 157.

Weil, R., 1917, *J. Amer. Med. Ass.*, **68**, 972.

Weiss, M., 1925, *Ann. Méd.*, **17**, 408.

Weyl, T., 1891, *Deuts. med. Wschr.*, **17**, 256.

Wildbolz, H., 1919, *KorrBl. Schweiz. Ärz.*, **49**, 793.

Willis, H. S., 1925^{1}, *Amer. Rev. Tuberc.*, **11**, 427; 1925^{2}, *ibid.*, **11**, 439; 1928, *ibid.*, **17**, 240.

Wolff-Eisner, A., 1907, *SitzBer. Berl. med. Ges.*, 15 May; 1908, *Beitr. Klin. Tuberk.*, **9**, 1; 1922, *Z. ImmunForsch.*, Tl. I, Orig., **35**, 215.

Zieler, K., 1910^{1}, *Arch. Derm. Syph.*, Wien, **102**, 37; 1910^{2}, *ibid.*, **102**, 257; 1911, *Deuts. med. Wschr.*, **37**, 2075.

ZIELER, K. & HÄMEL, J., 1926, *Beitr. Klin. Tuberk.*, **63,** 991.
ZIELER, K. & MARKERT, H. J., 1922, *Deuts. med. Wschr.*, **48,** 1672.
ZINSSER, H., 1921, *J. Exp. Med.*, **34,** 495.
ZINSSER, H. & MALLORY, T. B., 1924, *J. Immunol.*, **9,** 75.
ZINSSER, H. & MUELLER, J. H., 1925, *J. Exp. Med.*, **41,** 159.
ZINSSER, H. & TAMIYA, T., 1926, *J. Exp. Med.*, **44,** 753.
ZWICK, W. & TITZE, C., 1913, *Handb d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, Jena, **5,** 703.

The Laboratory Diagnosis of Tuberculosis.

BY J. MCINTOSH AND L. E. H. WHITBY.

WITH A SECTION BY A. STANLEY GRIFFITH.

The final diagnosis of any infection must depend on the successful demonstration of the causal microbe or virus, but in the case of tuberculosis, the finding of the tubercle bacillus with the microscope is often exceedingly difficult, and at times, impossible. The actual recognition of the tubercle bacillus presents little trouble to the skilled observer, and tuberculosis is one of the few diseases in which a diagnosis can be made by the microscope alone. The main difficulties arise from the relative frequency with which the bacilli are too few in number for direct demonstration, and from the fact that many lesions are closed, there being no outlet for the bacillus-containing exudate. The discharges are most likely to contain large numbers of bacilli when the tuberculous focus is caseous.

Where direct examination fails, either by the microscope, by culture or by animal inoculation, then there may be employed a number of adventitious methods, some of which depend on the immunity response.

The diagnosis of tuberculosis falls under the following headings :

(1) Demonstration of tubercle bacilli in excretions, discharges or tissues.

(2) Phenomenon of hypersensitiveness : tuberculin reactions.

(3) Presence of immune bodies : complement-fixation test ; agglutination.

(4) Non-specific phenomena : sedimentation of red cells ; blood- or cell-count ; tissue reaction.

DEMONSTRATION OF TUBERCLE BACILLI.

Though in the vast majority of cases the finding of tubercle bacilli in tissue or excretion indicates an active lesion, there are definite exceptions to this principle ; calcified material, long since inactive, may contain living tubercle bacilli in its centre ; tuberculous lesions healed and shut off with fibrous tissue may still harbour bacilli. On the other hand, the failure to find tubercle bacilli does not negative a positive clinical diagnosis ; fallacious is the sense of security given by one or even twenty negative reports on specimens of sputum, and, in many cases, the excretion of bacilli is so intermittent that whereas a specimen may be diagnosed at a glance on one day, on another the bacilli may be scanty or absent.

In the British Isles the question of other acid-fast bacilli confusing the issue rarely becomes of practical importance in human cases, but this possibility should be borne in mind in genito-urinary tuberculosis, and must always be considered in animal tuberculosis and when examining animal secretions, such as milk.

Methods of Demonstration.

Stained preparations.

At the present time the most commonly used method for staining tubercle bacilli is that of Ziehl-Neelsen (vide Vol. IX), and, like the majority of other methods, it is based on Ehrlich's discovery of the acid-fast nature of the microbe. Several modifications of Ziehl-Neelsen's method have been advocated, mainly involving the use of different counter-stains, but none appear to have any advantage except that 1 per cent. acid spirit is a cleaner decolorizing agent than 25 per cent. sulphuric acid, and is more pleasant to handle.

Other methods, such as that of Zirkowski, are satisfactory in the hands of those who have practised them, but it is an advantage to employ one method only, and to become expert with that rather than to ring the changes on a multitude of modifications.

It may be said with certainty that the finding of tubercle bacilli in a high proportion of specimens depends more on the experience and diligence of the observer and on his skill in the matter of selecting the most suitable material for his preparation than in the employment of fancy stains. A haphazard dip into a pot of sputum will frequently yield a negative result, when a carefully selected piece of purulent or caseous material from the same specimen will be loaded with tubercle bacilli.

Sputum. It is of the utmost importance to submit the specimen to a thorough macroscopic examination ; it should be poured from the container into a Petri dish and examined against a dark background. Smears should be made from purulent pieces, and especially from any yellowish nodules having the appearance of caseous material ; care should be taken not to mistake food particles for caseous material. Morning specimens and 24-hour specimens yield a higher proportion of positive results than do the scanty and chance expectorations of the day. Specimens consisting mainly of saliva or of thin mucus are usually, but not always, negative. Specimens containing a large amount of blood are usually negative, but are commonly positive once the hæmoptysis has ceased.

Concentration of the bacilli in the specimen has been advocated as an aid to diagnosis in cases where the bacilli are scanty. The procedure consists of adding a substance capable of dissolving the mucus, and so rendering the material liquid; the bacilli are then concentrated by centrifugalization. Uhlenhuth and Xylander (1908) recommended ' antiformin ' (liquor sodæ chlorinatæ and caustic soda) as the solvent, and Lange and Nitsche (1909) used ' ligroin ', a petrol-ether mixture, to concentrate the bacilli on the surface. These substances are undoubtedly an advantage with highly tenacious purulent specimens, the viscosity of which prohibits a thorough preliminary examination and the making of a good smear. Apart from this advantage general experience shows that, in ordinary cases, results do not justify the time expended on concentration methods.

Emphasis has already been laid on the necessity for making examinations of several specimens in sputum-negative cases with suggestive physical signs. Though repeated negative examinations by no means exclude tuberculosis, yet they should suggest the possibility of an alternative diagnosis. Corper (1928) estimates that a specimen of sputum must contain 100,000 bacilli per c.cm. before there is a reasonable chance of finding them in a stained film.

Some authorities have attempted to correlate the number of tubercle bacilli per field with the clinical findings, arguing that a decrease in bacilli should improve the prognosis. Whereas this would appear to be right in theory, it will be obvious that the number of bacilli is so largely dependent on the piece of material selected, that in practice the method must fail. The amount of sputum is a more accurate guide.

Only in exceptional cases is it worth while to spend more than ten minutes in examining one slide ; more fruitful results will be obtained from scrutinizing several daily specimens. It is preferable to find at least three typical tubercle bacilli before pronouncing a specimen positive. A positive slide should be broken and thrown away.

Patients with no apparent expectoration frequently swallow sputum ; especially is this the case with children. Sykes (1926) has estimated that an examination of the fæces for tubercle bacilli increases the probability of a positive diagnosis of pulmonary tuberculosis by about 9 per cent. The method should never be omitted in the case of children.

The importance of finding tubercle bacilli in the sputum before making a definite diagnosis of pulmonary tuberculosis may be gauged from the following table, which is abstracted from the report of the Chief Tuberculosis Officer for 1921 ; the table sets out the state of health of some 3,000 cases seven years after pulmonary tuberculosis had been diagnosed.

TABLE VIII.

	1921.		
	Unfit for Work.	Fit for Work.	Dead.
1914. With tubercle bacilli in sputum.	11·4 per cent.	42·3 per cent.	45·7 per cent.
1914. Without tubercle bacilli in sputum.	5·4 „	81·1 „	13·5 „

Commenting on the discrepancy in these figures, Kingston Fowler (1923) says: 'The figures are of no value at all except as a statement of the number of dead. The patients in one set were really suffering from pulmonary tuberculosis, and each of the others was no doubt suffering from some disease, but what it was no one can say'.

Urine. Other acid-fast bacilli, such as *B. smegmatis*, occasionally cause confusion, but no difficulty arises with a catheter specimen, or if an acid-alcohol decolorizing technique is adopted.

Cases of genito-urinary tuberculosis with active lesions excrete urine containing pus and tubercle bacilli; the bacilli may be sufficient in number to be found in a stained smear of the centrifugalized deposit, and are usually aggregated into characteristic clumps along with pus-cells. In early cases the bacilli are few and impossible to find; an acid urine with an occasional pus-cell may be the only finding even when clinical features are highly suggestive. Secondary infection with *B. coli*, streptococci, staphylococci or other pyogenic organisms frequently obscures a diagnosis of renal tuberculosis in that attention is paid only to the secondary organism. Animal inoculation should be performed in all doubtful or early cases.

Fæces. Tubercle bacilli are commonly found in fæces, but are only rarely an indication of tuberculous ulceration of the intestine. The bacilli are usually derived from swallowed sputum, particularly in the case of children. Examination of the specimen is greatly facilitated by the use of antiformin, ligroin or some other homogenizing substance.

Pus. The purulent material from a tuberculous abscess contains usually but few bacilli, but the appearance of the specimen is often characteristic. The thick, creamy pus, sometimes with small cretaceous particles, appears, in a stained smear, to be almost structureless and to consist largely of necrotic tissue-cells rather than leucocytes. Selection of caseous particles, as in the case of sputum, increases the chance of finding bacilli. Once the lesion has become open, secondary infection is common and the characteristic features of the tuberculous pus are obscured by the more acute pyogenic process. With tuberculous pus, and particularly if secondary infection is present, tubercle bacilli can usually only be demonstrated with certainty by animal inoculation.

Other specimens. It is uncommon to find tubercle bacilli in a pleural effusion unless the specimen is definitely turbid and contains pus. The clear straw-coloured effusions are usually negative to smear examination and animal inoculation. The same may be said of joint effusions. Tuberculous meningitis is a more rapid process, often of miliary type, and, as a general rule, the patient dies too soon for many tubercle bacilli to be present in the cerebrospinal fluid. The centrifugalized deposit sometimes shows bacilli in stained smears, but the nature of the disease is suggested by an increased cell-count, between 100 and 2,000, in which the predominant cell is usually a lymphocyte. Polymorphonuclear predominance occurs in early cases and in very acute cases; a reduction in the chloride content of the cerebrospinal fluid is said to favour the diagnosis.

Sections of tissue. Search for tubercle bacilli in sections of tissue is carried out as a sequel to histological examination. The finding of bacilli, though it confirms the characteristic histological changes (p. 303), frequently involves much labour in the cutting of serial sections. Before taking material for section from the respiratory passages, such as curettings from a buccal ulcer or tissue from the larynx, the site should be carefully swabbed to prevent the inclusion of adherent pieces of sputum.

Culture.

Culture of the tubercle bacillus direct from tuberculous material is rarely undertaken for diagnostic purposes owing to the length of time that must elapse before a growth is obtained. A description of the more suitable methods of cultivation will be found on page 162.

The advantage of a cultural method of diagnosis is that it is independent of virulence, a factor of considerable importance for successful animal inoculation. The development of a cultural method for obtaining growth with greater facility and certainty, would eliminate the expense and delay of animal inoculation experiments.

Acid-fast saprophytes appear quickly on artificial media and thus present few difficulties in differentiation.

Animal inoculation.

The fact that some of the lower animals are susceptible to tuberculosis affords a ready and simple means of detecting small numbers of tubercle bacilli. In a lower animal the production of tuberculosis is dependent on the type, the virulence and the number of bacilli inoculated, as well as on the species of animal used. The guinea-pig, the animal most used, is susceptible to both human and bovine types of tubercle bacillus, but somewhat more so to the latter type than to the former ; it is estimated by various authorities that from 10 to 160 bacilli of human type must be inoculated, the number varying with the virulence. Rabbits, although they are susceptible to a bovine infection, are very resistant to the human type ; they are, however, only used as a differential test.

When a guinea-pig is employed it is best to inoculate the material subcutaneously in the lower half of the anterior abdominal wall ; positive evidence of disease may be suspected when the animal shows loss of weight from week to week and when the inguinal glands can be detected on palpation. A post-mortem examination may be performed as soon as the inguinal glands are definitely enlarged, usually from 5 to 8 weeks after inoculation ; a stained smear of necrotic glandular material will usually contain tubercle bacilli ; miliary tuberculosis of greater or lesser extent is usual, the spleen being the organ most constantly affected. In view of the possibility of pseudo-tuberculous infections in laboratory animals it is essential to find tubercle bacilli in either smears or sections, and, should direct demonstration fail, then another animal must be inoculated with some of the post-mortem material. A guinea-pig may be expected to survive from about 6 weeks to 2 months after inoculation with a bovine strain, and from 3 to 9 months after inoculation with a strain of human type ; it is unnecessary to wait for the death of the animal in order to obtain a positive result. Intraperitoneal inoculation will reduce the period of waiting as the post-sternal glands are soon affected, but this procedure can only be used when the material is relatively free from contaminating bacteria.

Before inoculating an animal it is wise to make a Gram-stained smear and determine the amount of secondary infection. Specimens containing few or no secondary organisms may be inoculated without preliminary treatment. Specimens such as sputum and fæces, or samples of urine or pus containing streptococci, generally result in an acute septicæmia before sufficient time has elapsed for the tuberculous infection to be evident. Such specimens require preliminary treatment with an antiseptic that has not the power of penetrating the lipoid sheath of the tubercle bacillus, but which will kill the less well-protected pyogenic bacteria. Petroff's method (1915) is suitable for this purpose ; the sputum is shaken with an equal volume of 3 per cent. NaOH and then incubated for half an hour at 37° C. At the end of this time the mixture is made neutral to litmus with hydrochloric acid and then centrifuged ; some of the deposit thus obtained is used for inoculation. Antiformin or the glucoside ericolin, recommended by Twort (1910), may be used instead of caustic soda for sputum, fæces, urine or highly infected tissue ; the last traces of the antiseptic should be removed by washing before the deposit is inoculated. Corper (1928) states that 6 per cent. sulphuric acid is the best substance to use for killing off secondary organisms. It is often an advantage to make inoculations in duplicate, giving one animal a dose of the specimen direct, and the other the centrifugalized deposit from a large amount concentrated by the antiformin method. Pleural fluid, synovial fluid and ascitic fluid should be centrifugalized before inoculation and any clotted material should be included in the inoculum.

Tissue such as synovial membrane, omentum, tonsil or gland may be sewn into a subcutaneous pocket, but it is preferable to render the tissue fit for inoculation with a syringe and needle ; first it should be cut up into small pieces with scissors, then ground in a mortar with a little sand and 3 per cent. caustic soda. The fluid should then be placed in a test-tube to enable the coarse sand particles to settle out, which will occur in about five minutes ; the supernatant fluid containing the fine particles of tissue should be neutralized and inoculated subcutaneously.

Differentiation of the Types : Practical Considerations.

By A. Stanley Griffith.

In order to determine with certainty the type of infecting organism in a case of tuberculosis it is essential to isolate a culture. This may be obtained directly from the original material, with or without the aid of antiformin or potassium hydrate, or through the guinea-pig. It is always advisable to inoculate a guinea-pig subcutaneously with the material, not only for the purpose of obtaining a culture in the event of the direct cultures failing, but also for the diagnostic value of the test (see below).

The most easily prepared and the most reliable medium for primary cultures is plain inspissated egg, 3 or 4 tubes of which at least should be inseminated. In addition, glycerin (5 per cent.) egg tubes should be sown, since a comparison of the primary colonies on egg and glycerin egg gives useful preliminary information as to the type of the organism. If the colonies on egg are numerous (as is almost invariably the case when the inoculum is guinea-pig tuberculous tissue), transparent and filmy, while on glycerin egg they are sparse or absent, the strain is most probably bovine. When, on the other hand, the colonies on glycerin egg are as numerous as, and larger than, those on egg, the diagnosis is ' human ' if the colonies are dry and coherent, ' avian ' if the colonies are moist, smooth and easily emulsified.

Subcultures from the primary tubes should be made on egg or, preferably, on bovine serum, as soon as there is sufficient growth, i.e. after about 10 days to 3 weeks.

It is at this stage a good plan, which may save time and media, to make use of the other primary egg tubes in one or other of the following ways. If the preliminary diagnosis is ' bovine ' emulsify the growth of 1 or 2 tubes directly with a little salt solution, and inoculate the emulsion subcutaneously into a rabbit. It has been found that if the strain is a virulent bovine a small dose of the young and actively multiplying bacilli produces as rapidly fatal progressive tuberculosis in the rabbit as 10·0 mgm. of a subculture, and with as much constancy. Another of the primary cultures may be used to sow a potato tube ; eugonic human strains frequently produce on this medium their characteristic growths if the primary culture is young and actively growing.

The media which may be used for differentiating the types of tubercle bacilli are bovine serum, glycerin egg, glycerin serum, glycerin agar, glycerinated potato and glycerin broth. Of these bovine serum, glycerin egg or glycerin serum, and glycerinated potato are generally a sufficient variety ; glycerinated potato in the author's experience gives the sharpest differentiation between ordinary human, bovine and avian strains.

In these cultural tests one cannot insist too strongly on the importance of using young (that is, 5 to 8 days old) and vigorous subcultures from egg or serum. The growth from glycerin egg cultures should not be used for sowing the differential media, because dysgonic cultures are altered in character (become more eugonic) once having grown on media containing glycerin.

Standard human, bovine and avian strains are readily distinguished one from the other by cultural characters alone. Difficulty in classification arises in the case of strains which deviate from the standard types in cultural characters. Dysgonic human strains, for example, cannot be distinguished from bovine strains on glycerin agar, potato or broth. They, however, grow better than bovine strains on egg, serum, glycerin egg and glycerin serum and produce pigmented layers on suitable bovine serum. Avian strains may produce dry growths not easily distinguishable from those of mammalian tubercle bacilli and might therefore be wrongly classified if no animal tests were made.

While the determination of cultural characters enables one, with the above exceptions, to diagnose the type of a strain (or, in the case of mixtures, which is the predominant organism), the results of animal tests are less decisive. If one relied on virulence tests without reference to cultural characters for diagnosis of type there would be much confusion and error. For instance, the production of local tuberculosis only in the guinea-pig might be the result of infection with avian tubercle bacilli, or with mammalian tubercle bacilli which had become so reduced in virulence as to be incapable of producing progressive or generalized tuberculosis in the guinea-pig. Similarly, if a rabbit developed local or slight generalized tuberculosis after inoculation it would be impossible to decide whether the infecting bacilli were human, attenuated bovine, or avian, or mixtures of any of these types.

It is obvious from these examples that in order to ascertain whether a strain is a pure culture of human, bovine or avian bacilli, or whether it is an aberrant form of one of these types, cultural characteristics as well as virulence must be accurately established (see p. 164).

Table IX summarizes the cultural characteristics and pathogenic peculiarities of standard and atypical mammalian strains, and indicates how these several varieties may be differentiated one from the other.

Occasionally human and animal tuberculous tissues fail to cause visible lesions in a guinea-pig inoculated subcutaneously. While this result may indicate that there were no living tubercle bacilli in the material inoculated, it is not necessarily the case ; there may have been avian or attenuated mammalian bacilli present in extremely small numbers. In order to establish whether or not such a tuberculous lesion contained living tubercle bacilli, one must sow cultures from the inguinal glands of the guinea-pig, since avian bacilli, and also highly attenuated mammalian bacilli, can multiply in the guinea-pig at the seat of inoculation and in the adjacent glands without producing macroscopic lesions. The guinea-pig should be killed 6 to 8 weeks after inoculation, as after a time avian bacilli cease to multiply and die or are eliminated from the body.

Avian strains can generally be recognized in culture at an early stage of the investigation. For confirmation by virulence tests it is necessary to inoculate a fowl or a rabbit. Typical avian bacilli in very small number (0·001 mgm. or less) produce rapidly fatal tuberculosis in fowls inoculated intravenously. The most convincing evidence that a strain is avian is, however, the production of progressive tuberculosis by feeding, the bacilli being administered in the drinking-water of the fowl. Exceptionally, bacilli of avian type (from swine) exhibit low virulence for the fowl. In that event a rabbit should be inoculated intravenously with 5 to 10 mgm. of culture and kept under observation for 3 to 6 months. The development of joint lesions is evidence of the avian character of the strain.

TABLE X.

Cultural Characteristics.	Pathogenicity.			Diagnosis.
	Rabbits.		Guinea-pigs.	
	Intravenous 0·01 mgm.	Subcutaneous 10 mgm.	Intrap. or Subcut.	
Dysgonic on all media; no pigment on serum (also a few relatively eugonic).	Rapidly fatal G.M.T. within 5 weeks.	G.T. progressive and fatal usually within 10 weeks.	Rapidly fatal G.T. Lesions caseo-necrotic after subcut. inoc.	Standard bovine.
	G.M.T. varying in severity, usually fatal, sometimes retrogressive in character; duration of life longer than 6 weeks.	Effects ranging from G.T. of moderate severity, usually not fatal within 3 months, to local T. only.	Tuberculosis ranging from chronic G.T. with prolonged duration of life to slight retrogressive T.	Attenuated bovine.
(*a*) Eugonic on all media. (*b*) Eugonic on egg and serum, pigmented on latter; dysgonic on glycerin agar, potato and broth.	Lesions generally trivial, confined to lungs and (or) kidneys; often no T. at all.	Usually local lesion only.	Tuberculosis ranging from chronic G.T. with prolonged duration of life to slight retrogressive T.	(*a*) Attenuated eugonic human. (*b*) Attenuated dysgonic human
	Usually slight T. lungs and kidneys, not fatal within 3 months. Occasionally severe chronic G.T. Rarely rapidly fatal G.M.T.	T. ranging from local lesion to moderate G.T., severe in lungs only; not fatal within 3 months; disseminated lesions usually confined to lungs.	Rapidly fatal G.T., usually less caseo-necrosis and average duration of life longer than after standard bovine.	(*a*) Standard eugonic human. (*b*) Standard dysgonic human

G.M.T. = General miliary tuberculosis.

PHENOMENON OF HYPERSENSITIVITY.

Tuberculin Reactions.

The mode of action of tuberculin is now generally accepted as a phenomenon of hypersensitivity (allergy) depending on the sensitization of the tissues to the products of the growth of the tubercle bacillus. Tuberculin (O.T.) consists of the broken down protoplasm of cultivated bacilli and any product of the bacilli (B.E., T.R., &c.) will contain a certain amount of tuberculin. By the Regulations of the Therapeutic Substances Act (1927) a standard preparation of Old Tuberculin is kept at the National Institute of Medical Research, Hampstead, London.

The reaction may be general or local.

A perusal of the voluminous literature concerning the technique and the type of preparation that may be used for diagnostic purposes shows an appalling mass of contradiction, a multiplicity of methods and innumerable shades of opinion. Nowadays, Old Tuberculin (O.T.) is the only preparation that is at all extensively used for diagnostic work, and the technique is usually some modification of von Pirquet's original cutaneous method. The following are some of the methods that may be employed.

Subcutaneous method. This was the original method of Koch (1890, 1891) and causes local, focal and general reactions.

0·001 to 0·0001 c.cm. of O.T. are injected. The site of injection becomes inflamed and there is fever and obvious activity of any tuberculous focus; visible lesions, such as lupus, may become reddened or ulcerated, pulmonary cases may show increased cough and expectoration. The method is still used to a limited extent, but it is generally agreed that the danger resulting from the production of focal and general reactions outweighs the value of the positive evidence supplied by the test, and the procedure is now restricted to veterinary medicine.

Cutaneous method of von Pirquet (1907). The skin, usually of the flexor surface of the forearm, is cleansed with ether and two drops of Old Tuberculin are placed on the prepared surface about 4 inches apart. Nowadays, it is usual to use 50 per cent. or 25 per cent. tuberculin diluted with glycerin. The epidermis is scarified with a scalpel or sharp instrument, without drawing blood. Scarification is made first through a drop of glycerin at a control spot midway between the two drops of tuberculin, and then actually through the two drops. The tuberculin is allowed to act for 10 minutes and then wiped off. With a positive reaction an inflammatory redness and swelling appear around the site of inoculation, developing within 24 hours into a papule about half an inch in diameter. This reaches its maximum within 48 hours, and then gradually recedes. Such is a typical reaction, though slight and more intense reactions may be met with. In a negative reaction all three spots show merely a slight traumatic redness. A positive test supplies reliable evidence, probably absolute evidence, of a sometime tubercular infection, but, unfortunately, it does not differentiate a lesion active at the time of the test from an infection long since quiescent or overcome.

Believing that a more delicate method would go far towards solving the difficulty, Ellerman and Erlansden (1909, 1910) elaborated a quantitative technique known as the Quanti-Pirquet or qP. The method aims at distinguishing those persons who give a gross reaction even with minute quantities of tuberculin, from those who show only moderate reactions with comparatively large quantities. O.T. is used in geometrically progressive dilutions of 1 per cent., 4 per cent., 16 per cent. and 64 per cent. Scarification has to be skilfully and evenly performed and the papules resulting from each inoculation are carefully measured after 24 and 48 hours. A calculation as to sensitivity is made by reference to a series of tables elaborated by Erlansden (1911) which express the relation between the average papule size and the average difference in size between successive papules. Sensitivity figures above a certain point are said to indicate active disease. The method has been simplified by Morland (1912) and appears to be of value in the hands of skilled and experienced observers.

Intracutaneous method of Mantoux (1908, 1909). 0·1 c.cm. of dilutions of 1 in 1,000 and 1 in 10,000 of Old Tuberculin are injected into the cutis. A positive reaction is shown by redness and œdema and sometimes vesiculation, coming on within a few hours and reaching a maximum on the following day. The test is extremely sensitive, and on this account its use for clinical purposes is almost restricted to infants.

Method of Moro (1908). This consists of rubbing into the skin a paste made with equal parts of Old Tuberculin and lanoline. The inunction is usually made in the epigastric region and rubbing should be continued for about a minute. Reaction is evidenced by the appearance of papules in 24 hours.

The ophthalmo-reaction of Wolff-Eisner (1908) *and Calmette* (1907). The tuberculin used for instillation into the conjunctiva is a purified form of Old Tuberculin (1 per cent.). This test has been discarded completely owing to the fact that inflammatory reactions of disagreeable severity may occur, which may give rise to serious injury to the eye.

Value of Tuberculin Reactions.

Modern opinion inclines to the view that none of the tuberculin tests by themselves make a diagnosis absolute, except the subcutaneous method when it elicits a focal reaction. All tests may at times supply strong presumptive evidence, particularly when a negative reaction is obtained. It is generally believed that 80 per cent. of mankind have been infected with the tubercle bacillus at some time in their lives, and that only 1 out of every 3 or 4 has ever displayed the signs of active tuberculosis. Nevertheless, the sensitizing action of the infection persists, probably for life, and is manifested by tuberculin hypersensitivity. It is significant that large doses of tuberculin can be administered to new-born infants without any untoward result, the explanation being that tuberculin is innocuous to those who have never harboured the tubercle bacillus. In the first 2 or 3 years of life tubercular infection and disease are practically synonymous, and in early childhood the exhibition of a tuberculin reaction is very suggestive of active disease; the delicate cutaneous and intracutaneous methods are usually employed and the younger the child the greater is the weight that may be attached to a positive result.

In the adult, evidence of a positive reaction is rarely trustworthy until it has been balanced with the clinical findings and other evidence of disease. Much research has failed to reveal any certain method of correlating tuberculin sensitiveness of the skin and the activity of the disease, though, as a general rule, high tuberculin-sensitiveness and active disease go together. Marked reactions favour, but do not prove, active disease, and for this reason the quantitative reactions of Erlansden and others, appear to be a serious attempt to put the tuberculin test on a sound working basis.

A negative reaction in an adult practically eliminates a diagnosis of tuberculosis, though a negative result is not uncommon in the later stages of the disease, and, sometimes, after the absorption of a tuberculous pleural or peritoneal effusion.

The reliable general and focal reactions, so dangerous in human beings, can be used in veterinary medicine, and, as a consequence, the subcutaneous method of testing has been employed with great success.

DEMONSTRATION OF IMMUNE BODIES.

Complement-fixation Reaction in Tuberculosis.

In 1901, Bordet and Gengou discovered the phenomenon of fixation of complement, and, as a natural corollary, there followed the demonstration of antibodies in the sera of tuberculous human beings and animals by Widal and Le Sourd (1901), and Camus and Pagniez (1901). The work was followed up by Wassermann and Bruck (1906), who used Old Tuberculin as an antigen, and claim to have found antibodies in the sera of patients undergoing tuberculin treatment. Shortly afterwards, Citron (1907) stated that he had found similar antibodies in tuberculous patients who had not been so treated, though Morgenroth and Rabinowitsch (1907), and Meyer (1908), using a technique similar to Wassermann, obtained completely negative results. Nevertheless, Bermbach (1908), and Wolff and Mühsam (1908) were able to confirm Wassermann's results by finding 78 positive tests in 105 cases of pulmonary tuberculosis, whilst Cohn (1908) also demonstrated the reaction in 15 out of 57 cases.

About the same time, Wolff-Eisner (1908), Frugoni (1909), and, later, Caulfield (1911), Hammer (1912) and Fraser (1913) made use of emulsions of tubercle bacilli as an antigen, and, though the results showed some variation, yet in pulmonary tuberculosis from 62 to 97 per cent. of tests were found to be positive.

Early workers thus showed quite clearly that the reaction of fixation of the complement was likely to prove of some value as a clinical serological test for tuberculosis. It was recognized, however, that in human beings the amount of tubercular antibody was often small as compared with that present in immunized animals. In subsequent investigations considerable improvements in technique were introduced, and a variety of antigens were tried with a view to rendering the test more delicate. Standardization of all ingredients was undertaken ; Calmette and Massol (1911) standardized the complement ; they used a series of tubes with increasing amounts of complement and gave a quantitative indication of the strength of the antibodies present. On the other hand, McIntosh, Fildes and Radcliffe (1914) standardized the complement in the presence of the antigen, thus ensuring a more accurate titration. All authorities are now agreed that the hæmolytic amboceptor needs to be carefully titrated, and that the precise number of minimum hæmolytic units must be known ; the actual number of units advocated varies from 2½ to 12. It was soon evident to the majority of workers that a good antigen was imperative for the success of the test. Even to-day the question of the most suitable antigen has not been settled, and the value of many of the antigens exists only in the minds of their inventors. For the purpose of this résumé, the various antigens that have been used for the test may be placed in three groups :

(*a*) Tuberculin.
(*b*) Emulsions of tubercle bacilli.
(*c*) Extracts of tubercle bacilli, &c.

The vogue of tuberculin (old) was shortlived, for it was found to be insufficiently sensitive ; it has now quite gone out of use.

Undoubtedly, the most favoured antigen at the present time is some form of an emulsion of tubercle bacilli. The bacillary emulsion may be prepared in a number of ways. A young and rapidly growing culture on some special medium is usually employed. The bacilli may be used alive or they may be killed by heat or some other agent. The emulsion may be rendered more homogeneous by grinding the bacilli in some form of mortar (agate, Hayden's mill or a glass mill). Heat, according to some, has a deleterious effect on the antigen, and it is generally preferred to add an antiseptic such as phenol (McIntosh, Fildes and Radcliffe, 1914 ; Rogers, 1920). One of the most widely used bacillary antigens is that of Besredka (1913, 1921) ; for this, tubercle bacilli are grown for 4 or 5 days in Besredka's special egg broth ; the whole medium is then sterilized by heating at 100° C. for 30 minutes ; the bacilli are removed by centrifugalization and ground up in a glass mill. Before use, the antigen is standardized with a known tubercular serum. Other investigators have preferred extracts or macerations of tubercle bacilli. There are the alcoholic or acetone extracts of Dudgeon, Meek and Weir (1913), of Hammer (1912) and of Nègre and Boquet (1921), the glycerin extracts of Petroff (1917) and of Borrel and Boër (1920), the peptone extract of. Calmette and Massol (1914), whilst Wassermann, in 1923, introduced a tetraline extract of tubercle bacilli to which was added a small amount of lecithin. Like Wassermann, other workers have attempted to enhance the fixing power of the antigen by the addition of various lipoidal substances (cholesterol, &c.), but without any success; Schlossberger, Hartoch, Lusena and Prigge (1924) found that alcoholic, acetone, chloroform and tetraline extracts had all about the same antigenic power, whilst Coulthard (1923) states that most of these extracts are inferior to simple bacillary antigens.

Extracts of tuberculous tissues have been used by Meyer (1908) and Hammer (1912), but the specific antigenic power is apparently dependent on the richness of the tissues in tubercle bacilli (Debré and Paraf, 1911).

The multiplicity of antigens shows that many investigators believed that the perfecting of this reagent would solve the difficulty of sensitivity and result in a higher percentage of positive tests in proven cases of tuberculosis. It is believed that the fatty material contained in tubercle bacilli does not lend itself well to the fixation test, and, furthermore, larger doses of antigen cannot generally be used since it then becomes self-inhibitory and liable to yield non-specific results.

The optimum time for the completion of fixation is variously recommended as from 15 to 90 minutes, and some workers advocate the use of the water-bath, others the incubator. It is now known that fixation takes place rapidly, and Kahn and Johnson (1922) have shown that long incubation causes destruction of complement; prolongation of the time allowed for fixation must, therefore, tend to increase the chance of a non-specific result.

Specificity and scope of the test.

Many writers have indicated that the complement-fixation test is apt to give non-specific results, particularly in syphilis, malaria and leprosy. As early as 1906, Wassermann and Bruck noted that positive results were obtained with cases of syphilis and many have found that the non-specific reaction is more marked with some of the newer antigens, such as Besredka's, and with some of the extracts (Fried, 1924). McIntosh, Fildes and Radcliffe (1914) showed that bacillary emulsions, when properly standardized, do not tend to give non-specific results with Wassermann-positive sera. Nevertheless, it is not surprising that syphilitic sera show a tendency to react with tubercular antigens, for the latter contain a considerable amount of lipoidal substance of a like nature to that present in Wassermann syphilitic antigen. The addition of lecithin or cholesterol to the tubercular antigen must, of necessity, tend to increase the non-specific factor, and, in the case of Besredka's antigen, extraneous lipoid is probably derived from the egg medium. Silberstein (1924) states that Wassermann's tetraline antigen has considerable non-specific properties. In leprosy, the frequency of positive results with tubercular antigens was shown by Slatineanu and Danielopol (1908), McIntosh, Fildes and Radcliffe (1914), Taylor and Malone (1924) and others. This result is to be expected in view of the close relationship between the tubercle and lepra bacilli. In malaria, positive non-specific results have been recorded by Rieux and Bass (1921) and Heinemann (1921), but others have failed to confirm this. Lastly, it is quite definite that the complement-fixation test in tuberculosis has only a group specificity ; tubercular sera will give a positive reaction with antigen composed either of bacillary emulsions or of extracts of bovine or human tubercle bacilli, as well as with other types of acid-fast bacteria, including the lepra bacillus, Coulthard (1923), Osumi (1924).

The complement-fixation reaction has been used in all forms of clinical tuberculosis but has given the best results in pulmonary cases. Nevertheless, a good proportion of other cases such as joint infections, adenitis, peritonitis, meningitis and lupus yield positive results.

Reaction in normal individuals. The sera of a fair number of apparently healthy individuals have been tested for tuberculosis by the complement-fixation test and somewhat divergent results have been obtained. Urbain (1925) compiled a list of some 2,000 non-tuberculous patients and found that 93 per cent. were negative. The average positive finding in apparently normal persons is from 4 to 10 per cent. One of the writers (J.M.) examined some 300 university students and found 6 per cent. gave positive reactions. For this and other reasons, it is important always to include a positive control serum when carrying out the test.

Review of results. In view of the multiplicity of techniques, methods and reagents, it is difficult to make an accurate general estimate of the results of the test. The figures of one observer can hardly be compared with those of another when their methods are so different, and very few observers appear to have followed one technique in performing a large series of tests. The results of some authorities are shown in Table X, and an estimate of the average findings in some 30,000 cases is set out beneath.

Summary : diagnostic value of the test.

The literature and statistics relating to the complement-fixation test for tuberculosis show clearly that the results have never given an agreement with clinical findings at all comparable in reliability with the Wassermann reaction in syphilis. It is true that the test for tuberculosis cannot be judged as strictly as the Wassermann test, since syphilitic infection is relatively rare compared with the number of apparently normal individuals that have, at one time or another, contracted some form of tubercular infection. The reaction for tuberculosis, therefore, needs careful adjustment so that only a very definite concentration of antibody will give a positive result. But although particular attention to technique and to the antigen have given better results, there is still considerable room for improvement.

Table X summarizes the average experience of different workers, and shows that the complement-fixation test fails to detect tuberculosis in some 16 per cent. of clinically and bacteriologically proven cases, and that it indicates tuberculosis, past or present, in 4 to 10 per cent. of apparently normal controls.

TABLE X.

Authors.	Results.
Alcock, Douglas and Lucey (1925) ..	90·6 per cent. in bacillus-positive cases. 36·5 per cent. in bacillus-negative cases.
Calmette and Massol (1914)	92 per cent. in proven cases.
Caulfield (1911)	56 per cent. in proven cases.
Cooke (1921)	80 per cent. in children.
Delille, Hillemand and Lestocquoy (1922).	85 per cent. in bacillus-positive cases.
Dudgeon, Meek and Weir (1913) ..	84 to 89 per cent. in pulmonary cases. 57 per cent. in surgical cases.
Fidlar (1918)	74 to 77 per cent. in pulmonary cases.
Fraser (1913)	68 per cent. in bacillus-positive cases.
Fried (1920)	94 per cent. in pulmonary cases.
Hruska and Pfenniger (1921)	84·5 per cent. in animals (proved *post mortem*). 2·2 per cent. in controls.
Ichok, Goldenburg and Fried (1920) ..	66·4 per cent. in cases of lupus. 16·3 per cent. negative in cases of lupus.
Kalcher and Sonnenfeld (1924).. ..	60 per cent. in clinically positive cases.
McIntosh, Fildes and Radcliffe (1914)..	76·7 to 90 per cent. in pulmonary cases. 80·7 per cent. in surgical cases.
Pai and Rau (1927)	96·7 per cent. in clinically positive cases. 13 per cent. in non-tuberculous cases.
Pinner (1927)	69 per cent. in acute cases. 63 per cent. in inactive cases. Normal sera, 15 per cent. Wassermann-positive, 29 per cent.
Punch (1921 and 1920)	98 to 100 per cent. in pulmonary cases.
Punch and Gosse (1922)	98 per cent. in pulmonary cases.
Urbain and Fried (1921)	35 per cent. of syphilitics gave a positive.
von Wedel (1920)	70 per cent. in clinically positive cases.
Wang and Crockett (1919)	84 to 87 per cent. in proven cases.
Watkins and Boynton (1920)	64·6 per cent. in proven cases. 4·2 per cent. in non-tuberculous cases.

Average Findings (from literature):

For pulmonary proven cases..	83·5 per cent.
For clinically positive pulmonary cases	64 ,,
For surgical cases	68 ,,
In normal controls	4 to 10 ,,
With Wassermann-positive	30 to 40 ,,

Added to this is the fact that a syphilitic patient with a strong Wassermann reaction will, in a small proportion of cases, give a positive complement-fixation reaction for tuberculosis. Furthermore, positively reacting tuberculous sera will, at times, fix complement equally well with an antigen of acid-fast bacteria other than tubercle bacilli (Dienes and Balas, 1924), or even with such entirely unrelated bacteria as *B. subtilis* and *C. diphtheriæ* (Ogawa, 1925).

In these circumstances the reliability of the test even for qualitative purposes must be questioned; attempts to put the reaction on a quantitative basis have entirely failed to supply any reliable index as to cure, prognosis or activity. There is no doubt that a single negative test is quite useless for excluding tuberculosis, in the same way that a single negative Wassermann reaction does not exclude syphilis, or a Widal, typhoid fever. In its present position the test must be regarded as confirmatory rather than primarily diagnostic. It is essential to perform a Wassermann reaction at the same time.

The moderate degree of success obtained by this test is possibly explained by the insignificant part that humoral antibodies play in tuberculosis, particularly in encapsuled and localized lesions unaccompanied by a general reaction. It is unknown to what extent complement fixers indicate resistance; they may be found in tuberculous persons showing no clinical evidence of disease; they are frequently absent in the very advanced stages of the disease, and also in incipient and minimal lesions poor in signs and symptoms; they are but 'witnesses of infection' (Calmette) and are not dependable as such.

For these reasons, therefore, it is doubtful whether an organized investigation correlating the results of experienced serologists would succeed in putting the test on the same firm basis as the Wassermann reaction.

Test in veterinary medicine.

The complement-fixation test has, in veterinary medicine, been applied to the diagnosis of tuberculosis, particularly in cattle and dogs, but the results have been variable and distinctly discouraging. Thus, Calmette, Massol and Breton (1908) found only 50 per cent. positive and Bierbaum and Berdel 65 per cent.; on the other hand, Ruppel and Rickman (1910), Brocq-Rosseu, Urbain and Cauchemez (1923) and Panisset and Verge (1922) obtained positive results in from 80 to 94 per cent.

The great variability in the above figures is somewhat surprising considering that the tests were made, in most instances, on animals known to be suffering from tuberculosis, as demonstrated at autopsy or by tuberculin. It is little wonder, therefore, that the test has gained no ground as a practical method in veterinary medicine.

Agglutination.

The agglutination reaction has been applied to the diagnosis of tuberculosis with somewhat indefinite results. An almost insurmountable difficulty is the great tendency of an emulsion of tubercle bacilli to undergo spontaneous clumping. Arloing (1898) claimed to have overcome this by the use of his homogeneous emulsions, obtained by growing the bacilli in cultures constantly agitated. Other homogenizing procedures have been advocated, such as by grinding the bacilli in a mortar.

Arloing and Courmont (1900) obtained positive reactions in 87·9 per cent. of certain cases of tuberculosis and 34·6 per cent. in suspected cases, but, unfortunately, 26·8 per cent. of apparently healthy individuals gave positive results. Other observers have failed to confirm these findings, and state that, as a diagnostic measure, agglutination for tuberculosis is of no value.

Similarly, the precipitin reaction has been found to be of no practicable value.

NON-SPECIFIC PHENOMENA.

Sedimentation of Red Corpuscles.

For more than a century it has been known that the separation of blood into plasma and corpuscles is, in some pathological conditions, remarkably rapid, and to Westergren (1921) is due the credit of applying the phenomenon as an aid to the diagnosis of tuberculosis. Westergren investigated some 5,000 samples of blood and standardized a test which he named 'The suspension stability rate of the erythrocytes'. The test is now usually known as the 'Sedimentation Test' or 'Sedimentation Rate'. The principle of all methods is briefly as follows: A column of citrated blood is allowed to stand and separate into plasma and corpuscles; standard volumes of sodium citrate solution and of blood are used and the degree of sedimentation is observed at the end of stated times, 1 hour, 2 hours or 24 hours; the amount of supernatant plasma or of underlying corpuscles gives the measure of sedimentation. Observations may be made until sedimentation is complete and the result expressed as rate per hour, or, as in Westergren's original technique, plotted as a curve at stated hours; nowadays, the degree of sedimentation at the end of one hour is considered to furnish all the information required.

For performing the test many different methods have been advocated, but all fall into one of two classes. Westergren's (1921) original technique is an instance of what may be called the 'capillary tube' method, in which the citrated blood is placed in a capillary tube of standard bore and length; the result is expressed as the length of the plasma column after standing for 1 hour, but observations may be continued for a period of 24 hours. (Amounts: 0·2 c.cm. of 3·8 per cent. sodium citrate plus 1 c.cm. blood; 1 c.cm. of mixture placed in a capillary tube of length 200 mm. with capacity of 1·0 c.cm.)

More reliable are the methods which require a larger volume of blood and a larger tube and in which the result is expressed in corpuscle or plasma volume. Thus Zeckwer and Goodell (1925) recommend placing 10 c.cm. of citrated blood into a 15 c.cm. centrifuge tube graduated in tenths of a c.cm.; after standing for 1 hour the volume of corpuscles is read. (Amounts: 2 c.cm. of 3 per cent. sodium citrate, 8 c.cm. of blood.)

German investigators, such as Linzenmeier (1922), use a similar technique, but express the result by the volume of supernatant plasma.

The degree of sedimentation has been found to be increased in all conditions in which there is tissue destruction: in such conditions as acute infections, active tuberculosis and some forms of malignant disease. The phenomenon is said to be due to an increased fibrinogen content of the blood.

Results and interpretations.

Zeckwer and Goodell (1925) give the following average figures for normal persons and certain pathological conditions, when using 10 c.cm. of citrated blood :

	Volume of Red Cells at end of 1 Hour
Normal	7 c.cm.
Pregnancy	6 ,,
Malignant disease	4 ,,
Tuberculosis	4·4 ,,
Acute inflammation	4·25 ,,

General experience shows that the figure of 7 c.cm. for the average normal is too small ; 8·5 to 9 c.cm. is more common. Moreover, in a known case of tuberculosis a figure of 7 c.cm. would suggest activity.

There is no sharp demarcation between normal and pathological cases and there are well-marked differences between individuals irrespective of a pathological condition ; yet the result is remarkably constant for a particular person in health. Sedimentation is more complete in women than in men and the phenomenon is more marked at the menses as well as during pregnancy.

The sedimentation test is, therefore, limited in usefulness ; it is in no way specific and should not be used apart from careful clinical investigation ; it is practically essential to establish a diagnosis of tuberculosis by other methods before considering the evidence afforded by the sedimentation test. The most reliable information is supplied not from one single test but from a series performed at varying periods.

Value in tuberculosis.

As a general rule the degree of sedimentation is increased in active pulmonary tuberculosis and more so in the caseating and exudative type than the cirrhotic. Võlckers (1924), and others have found the test of no value in surgical tuberculosis. Sedimentation, like temperature, would appear to be some measure of toxæmia and activity ; Tegtmeier (1923) considers that an increase in sedimentation often precedes clinical evidence of progress of the disease. Any attempt to use the test as diagnostic only results in the most grave errors, but as a supplement to skilled clinical, radiological and general examination it can be of value. Particularly is this so when a series of observations are made on individual cases ; the degree of sedimentation follows closely the clinical improvement or retrogression ; figures of 3 or 4 c.cm. may be found in highly active cases improving to 8 or 9 c.cm. with the subsidence of activity. An alteration towards normal is of good prognosis.

General experience indicates that, provided the limitations of the test are realized, then it is of some value in estimating the activity or arrest of pulmonary tuberculosis and gives some evidence on which to base a prognosis.

The value of the test has been investigated by Fischel (1925), Gripenberg (1925), Papanikolau and Weiller (1925), Rennebaum (1923), Alterthum (1923), Schellenburg and Naucke (1923) and Krimphoff (1923).

Cellular Reaction.

Blood, exudates, fluids and tissue.

The chronic nature of most tuberculous infections is shown by the occurrence of leucopenia with relative lymphocytosis; polymorphonuclear leucocytosis, both absolute and relative, is found in miliary conditions, but is more often suggestive of secondary infection with pyogenic bacteria. Anæmia of secondary type is common and though usually of mild degree, yet in severe cases it may be well marked. The cellular reaction to tubercular infection is characteristically lymphocytic, though polymorphonuclear predominance may occur with acute lesions. Hence, inflammatory exudates, such as pleural, ascitic or synovial fluids contain mainly lymphocytes and these in comparatively small numbers; frankly purulent fluids suggest rather a secondary infection.

In tuberculous meningitis the cellular content of the cerebrospinal fluid is increased and the total number of cells per c.mm. may be from a hundred to a few thousands. Lymphocytic predominance is the rule, but usually 25 to 45 per cent. of the cells are polymorphonuclears; in young children the polymorphonuclear content may reach as high as 70 to 80 per cent.

In a certain number of instances the diagnosis of tuberculosis will depend on the microscopical appearances of the diseased tissues, particularly with lesions of the skin, glands and joints. In such lesions the tubercle bacilli are few in number and difficult to demonstrate by the microscope, so that where time is important, reliance has to be placed on the histological appearance. Where practicable, confirmation should be sought by inoculating some of the tissue into a guinea-pig.

The recognition of the lesion is dependent on the appearance of the tubercular granuloma, a matter of no great difficulty in chronic cases. The typical giant-cell systems with peripheral fibrosis, central caseation and lymphocytic infiltration are, in most cases, sufficiently characteristic.

REFERENCES.

ALTERTHUM, L., 1923, *Beitr. Klin. Tuberk.*, **55,** 511.

ARLOING, S., 1898, *C.R. Acad. Sci.*, Paris, **126,** 1319 and 1398.

ARLOING, S. & COURMONT, P., 1900, *Z. Tuberk.*, **1,** 116.

ARMAND-DELILLE, P., HILLEMAND, P. & LESTOCQUOY, C., 1922, *Bull. Soc. méd. Hôp. Paris*, **38,** 883.

BERMBACH, P., 1908, *Z. Tuberk.*, **13,** 193.

BESREDKA, A., 1913, *C.R. Acad. Sci.*, Paris, **156,** 1633; 1921, *Ann. Inst. Pasteur*, **35,** 291.

BIERBAUM, K. & BERDEL, G., 1914, *Z. ImmunForsch.*, **21,** 249.

BORDET, J. & GENGOU, O., 1901, *Ann. Inst. Pasteur*, **15,** 289; 1903, *C.R. Acad. Sci.*, Paris, **137,** 351.

BORREL, A. & BOËR, L., 1920, *C.R. Soc. Biol.*, Paris, **83,** 1130.

BROCQ-ROSSEU, A., URBAIN, A. & CAUCHEMEZ, 1923, *Ann. Inst. Pasteur*, **37,** 872.

BROUGHTON-ALCOCK, W., DOUGLAS, M. & LUCEY, H. C., 1925, *Lancet*, Lond., i, 1331.

CALMETTE, A., 1907, *C.R. Acad. Sci.*, Paris, **144,** 1324 ; 1920, *L'infection bacillaire et la tuberculose*, 1st ed., p. 467, Paris (Masson et Cie).

CALMETTE, A. & MASSOL, L., 1911, *C.R. Soc. Biol.*, Paris, **71,** 191 ; 1914, *Ann. Inst. Pasteur*, **28,** 338.

CALMETTE, A., MASSOL, L. & BRETON, M., 1908, *C.R. Acad. Sci.*, Paris, **146,** 676.

CAMUS, J. & PAGNIEZ, P., 1901, *C.R. Soc. Biol.*, Paris, **53,** 734.

CAULFIELD, A. H., 1911, *J. Med. Res.*, **24,** 101.

CITRON, J., 1907, *Berl. klin. Wschr.*, **44,** 1135.

COHN, S., 1908, *Berl. klin. Wschr.*, **45,** 1309.

COOKE, J. V., 1921, *Amer. J. Dis. Chil.*, **21,** 78.

CORPER, H. J., 1928, *J. Amer. Med. Ass.*, **91,** 371.

COULTHARD, H. L., 1923, *J. Path. Bact.*, **26,** 350.

DEBRÉ, R. & PARAF, J., 1911, *C.R. Soc. Biol.*, Paris, **71,** 359.

DIENES, L. & BALAS, L., 1924, *Amer. Rev. Tuberc.*, **9,** 144.

DUDGEON, L. S., MEEK, W. O. & WEIR, H. B., 1913, *Lancet*, Lond., i, 19.

ELLERMANN, V. & ERLANDSEN, A., 1909, *Deuts. med. Wschr.*, **35,** 436 ; 1910, *Beitr. klin. Tuberk.*, **16,** 1.

ERLANDSEN, A., 1911, *Beitr. klin. Tuberk.*, **18,** 419.

FIDLAR, E., 1918, *Lancet*, Lond., ii, 844.

FISCHEL, K., 1925, *Amer. Rev. Tuber.*, **10,** 606.

FRASER, E. T., 1913, *Z. ImmunForsch.*, Tl. I, Orig., **20,** 291.

FRIED, B., 1920, *C.R. Soc. Biol.*, Paris, **83,** 1312 ; 1924, *Amer. Rev. Tuberc.*, **9,** 112.

FRUGONI, C., 1909, *Berl. klin. Wschr.*, **46,** 1724.

GRIPENBERG, R., 1925, *Acta tuberc. scand.*, **1,** 121.

HAMMER, C., 1912, *Münch. med. Wschr.*, **59,** 1750.

HEINEMANN, H., 1921, *Münch. med. Wschr.*, **68,** 1551.

HRUSKA, C. & PFENNINGER, W., 1921, *Ann. Inst. Pasteur*, **35,** 96.

ICHOK, C., GOLDENBERG, L. & FRIED, B., 1920, *C.R. Soc. Biol.*, Paris, **83,** 1363.

KAHN, R. L. & JOHNSON, S. R., 1922, *J. Infect. Dis.*, **31,** 416.

KALCHER, H. & SONNENFELD, A., 1924, *Z. Tuberk.*, **40,** 420.

KINGSTON FOWLER, J., 1923, *Problems in Tuberculosis*, p. 24, Oxford Medical Publications, 1st ed.

KOCH, R., 1890, *Deuts. med. Wschr.*, **16,** 1029 ; 1891, *ibid.*, **17,** 101 & 1189.

KRIMPHOFF, F., 1923, *Beitr. Klin. Tuberk.*, **55,** 365.

LANGE, L. & NITSCHE, P., 1909, *Deuts. med. Wschr.*, **35,** 435.

LINZENMEIER, G., 1922, *Deuts. med. Wschr.*, **48,** 1023.

MCINTOSH, J., FILDES, P. & RADCLIFFE, J. A. D., 1914, *Lancet*, Lond., ii, 485.

MANTOUX, CH., 1908, *C.R. Acad. Sci.*, Paris, **147,** 355 ; 1909, *C.R. Soc. Biol.*, Paris, **66,** 54.

MEYER, K., 1908, *Deuts. med. Wschr.*, **34,** 868.

MORGENROTH & RABINOWITSCH, L., 1907, *Deuts. med. Wschr.*, **33,** 705.

MORLAND, E. C., 1912, *Lancet*, ii, 688.

MORO, E., 1908, *N.Y. Med. J.*, **87,** 1233 ; *Pr. méd.*, Paris, **16,** 484.

NÈGRE, L. & BOQUET, A., 1921, *Ann. Inst. Pasteur*, **35,** 300.

OGAWA, T., 1925, *Z. ImmunForsch.*, **43,** 339.

OSUMI, S., 1924, *Z. ImmunForsch.*, **40,** 237.

PAI, M. K. & RAU, K. VENKATA, 1927, *Tubercle*, Lond., **8,** 366.

PANISSET, L. & VERGE, J., 1922, *Ann. Inst. Pasteur*, **36,** 690.

PAPANIKOLAU, B. & WEILLER, P., 1925, *Paris méd.*, **46,** 400.

PETROFF, S. A., 1915, *J. Exp. Med.*, **21,** 38 ; 1917, *Amer. Rev. Tuberc.*, **1,** 33 ; 1920, *ibid.*, **3,** 683.

PINNER, M., 1927, *Tubercle*, Lond., **8,** 415.

PIRQUET, C. VON, 1907, *Berl. klin. Wschr.*, **44,** 699.

PUNCH, A. L., 1920, *Lancet*, Lond., ii, 647 ; 1921, *ibid.*, ii, 497.

PUNCH, A. L. & GOSSE, A. H., 1922, *Brit. Med. J.*, i, 509.

RENNEBAUM, H., 1923, *Beitr. Klin. Tuberk.*, **57,** 263.

RIEUX, J. & BASS, 1921, *Ann. Inst. Pasteur*, **35,** 378.

ROGERS, J. B., 1920, *J. Infect. Dis.*, **27,** 101.

RUPPEL, W. G. & RICKMAN, W., 1910, *Z. ImmunForsch.*, Tl. I, Orig., **6,** 344.

SCHELLENBURG, G. & NAUCKE, H., 1923, *Beitr. Klin. Tuberk.*, **57,** 81.

SCHLOSSBERGER, H., HARTOCH, O.. LUSENA, M. & PRIGGE, R., 1924, *Deuts. med. Wschr.*, **50**, 869.
SILBERSTEIN, S., 1924, *Deuts. med. Wschr.*, **50**, 675.
SLATINEANU, A. & DANIELOPOL, D., 1908, *C.R. Soc. Biol.*, Paris, **64**, 772.
SYKES, K. A. H., 1926, *Lancet*, Lond.. i, 273.
TAYLOR, J. & MALONE, R. H., 1924, *Ind. J. Med. Res.*, **12**, 127.
TEGTMEIER, R., 1923, *Deuts. med. Wschr.*, **49**, 1113.
TWORT, F. W., 1910, *Proc. R. Soc. Med.*, **3**, 57.
UHLENLUTH & XYLANDER, 1908, *Berl. klin. Wschr.*, **45**, 1346.
URBAIN, A., 1925, *La réaction de fixation dans la tuberculose*, 1st ed., p. 103, Paris (Masson et Cie).
URBAIN, A. & FRIED, B., 1921, *Ann. Inst. Pasteur*, **35**, 294.
VÖLCKERS, C., 1924, *Beitr. Klin. Tuberk.*, **57**, 359.
WANG, C. Y. & CROCKET, J., 1919, *Brit. Med. J.*, ii, 7.
WASSERMANN, A. VON, 1923, *Deuts. med. Wschr.*, **49**, 303.
WASSERMANN, A. VON & BRUCK, C., 1906, *Deuts. med. Wschr.*, **32**, 449.
WATKINS, W. W. & BOYNTON, C. N., 1920, *J. Amer. Med. Ass.*, **75**, 933.
WEDEL, H. O. VON, 1920, *J. Immunol.*, **5**, 159.
WESTERGREN, A., 1921[1], *Acta med. scand.*, **54**, 247 ; 1921[2], *Brit. Tuberc.*, **15**, 72.
WIDAL, F. & LE SOURD, L., 1901, *Bull. Soc. méd. Hôp. Paris*, **18**, 624.
WOLFF-EISNER, A., 1908, *Berl. klin. Wschr.*, **45**, 65.
WOLFF, M. & MÜHSAM, H., 1908, *Deuts. med. Wschr.*, **34**, 1504.
ZECKWER, I. T. & GOODELL, H., 1925, *Amer. J. Med. Sci.*, **169**, 209.

Treatment.

TUBERCULIN.

BY A. FLEMING.

Tuberculin was introduced by Robert Koch (1890) for the treatment of tuberculous infection in man, not as an immunizatory agent, but as a toxic product which had a specific affinity for tuberculous tissues and induced an inflammatory reaction in such tissues, leading sometimes to necrosis and sloughing. His original system of dosage was based on this assumption, that focal reactions were essential to the success of the treatment. As the science of immunology advanced, however, it was shown that the injection of tuberculin induced the elaboration of specific antibodies, and all the later work on the subject has had specific immunity as its basis.

An enormous number of different ' tuberculins ' have been produced for use in the treatment of tuberculosis, and it would be out of place here to consider them in detail. They can be divided into five main classes : (1) filtrates of old broth cultures, frequently concentrated ; (2) suspensions of tubercle bacilli ; (3) tubercle bacilli which have been subjected to some process to extract an undesirable element ; (4) living attenuated tubercle cultures ; (5) similar preparations made from cultures of tubercle bacilli other than the human type.

The Therapeutic Substances Act has limited the name ' tuberculin ' to the first group and lays down that the name ' Tubercle Vaccine ' shall be used for preparations of the actual bacilli. This nomenclature is useful in that the main active principle of ' Tuberculin ' can be standardized (see p. 309), whereas, although ' Tubercle vaccines ' may contain some of the same active principle, the other antigens cannot be standardized in any way other than the indirect method of estimating the weight of dried bacilli or the number of bacilli per unit of fluid.

The problems in the treatment of tuberculosis by vaccines are essentially the same as in other infections, except that the histological formation of the tubercle lesion is not such as would conduce to a rapid result being obtained by any immunizing process. The fact, too, that many bacilli are contained in necrotic material secluded from the blood supply, introduces another serious complication, for Wright and Douglas (1904) have shown that in such ' non-bacteriotropic niduses ' the antibodies (opsonins) are much reduced or absent, so that, however great is the general immunity produced, some of the bacteria are almost beyond the reach of the protective substances.

Just as in other infections it is impossible to adjust the therapeutic doses of tubercle vaccine by observations on healthy individuals for the following reasons :

1. The tuberculous patient has acquired a hypersensitiveness to the products of the tubercle bacillus. This is evidenced by the ophthalmo, cutaneous, subcutaneous and other tests used for diagnosis.

2. The patient may be auto-inoculating himself, i.e. he may be absorbing an unknown amount of tuberculous material from his infected focus. Wright and others have shown that the changes in the opsonic index to tubercle are the same following disturbances of the infected focus as they are after the injection of tubercle vaccine.

In the treatment of any tuberculous lesion, therefore, by means of vaccine it is necessary to keep the infected portion of the body as nearly as possible at rest. In the case of tuberculous arthritis this may be done efficiently by splinting, but in pulmonary tubercle it may be impossible, although much can be done to prevent auto-inoculations by absolute rest or by artificial pneumothorax, complete or partial.

In the consideration of the treatment of tuberculous infections a sharp line must be drawn between the use of filtrates of broth cultures (tuberculins) and preparations of the bacilli themselves (tubercle vaccines). The most important constituent of the former is a toxic body or antigen quite distinct from the antigens of the bacillary body. When, therefore, tuberculin is used solely in the treatment of a tubercular infection, it can only be expected that an immunity will be produced against tuberculin or that the patient will be desensitized to this substance. It would be unreasonable to assume that a complete immunity would be produced against the tubercle bacillus. On the other hand, with tubercle vaccines immunity would be produced against the bacilli, although there might be little or no immunity against the toxic agent contained in tuberculin, and little or no desensitization to tuberculin. It is an old observation that individuals who have recovered from a tuberculous infection remain sensitive to tuberculin, as evidenced by the von Pirquet and other tests.

Following Koch's first publications on the subject, the use of tuberculin as a toxi-therapeutic agent for the treatment of tuberculosis in man rapidly spread throughout the world. The violence of the reactions, however, and the not infrequent generalization of the disease as a result of the administrations caused it to be largely abandoned.

The first era of tuberculin treatment, therefore, was one of large doses. In the more modern treatment two main schemes of dosage have been adopted. The first system, which is chiefly adopted by the continental physicians (see Bandelier and Roepke, 1913, who give a bibliography), is one in which the original dose is so small that no reaction is induced, and the doses are repeated at short intervals and increased as rapidly as can be done without reactions occurring, until very large amounts can be administered. Sometimes tubercle vaccines are used alone in this scheme, or sometimes old tuberculin is also given, the aim then being to establish an immunity to the toxins as well as to the tubercle bacillus. This system of rapidly increasing doses is very similar to the scheme at present adopted in the desensitization of patients suffering from hay fever and other allergic conditions.

The second system owes its inception to the work of Wright and his followers on the changes in the opsonic content of the serum following the inoculation of tubercle and other vaccines. They showed that a minute dose of tubercle vaccine induced a rise in the opsonic index without any preliminary depression, a larger dose would be followed by a transient depression of the opsonic index, after which there would be a considerable rise, while a very large dose induced a profound and lasting depression in the opsonic content. On this basis their aim was to administer, as far as possible, such quantity as would substantially increase the opsonic power without first seriously depressing it, in other words to obtain the maximum positive phase with the minimum negative phase. While such is the ideal it is in practice almost impossible to attain, and a compromise has in each to be adopted. If a patient is so seriously ill that a set-back, even a slight one, might have serious results, then the dose must be so small that there is no negative phase. In such a case, however, the positive phase is small and transient and the vaccine has to be frequently repeated. On the other hand, if the patient has a small circumscribed lesion it is permissible to administer a larger dose, which, although it may temporarily induce a negative phase, will be followed by a more pronounced and prolonged positive phase. For this reason no hard and fast scheme of dosage can be laid down for all cases.

Following Wright's system of using tubercle vaccines (see Wright, 1909) the initial dose in cases of localized tubercle (e.g. arthritis or adenitis) is about 0·00001 mgm. and the doses are repeated every 4 to 7 days. In many cases the amount administered is never greater than 0·0001 or 0·0002 mgm. There is no reaction, either focal or general, following the administrations of the vaccine. In the more localized forms of tubercle such as adenitis, arthritis or pyelitis, this system of treatment has given excellent results (Maynard Smith, 1909), but in pulmonary tubercle the results have not been so favourable, more especially in those cases where the auto-inoculations could not be controlled. In all cases, Wright has insisted on the elimination, so far as is possible, of all non-bacteriotropic niduses and also that when there are secondary infections these should be treated with appropriate vaccines.

Some constituents of the tubercle bacillus have been shown (see p. 224) to be especially toxic for animals, and many attempts have been made to eliminate the toxic elements or to isolate them and replace them in smaller amounts. None of the preparations so made have had, in practice, any striking advantage over preparations of the whole bacilli. Douglas (1921) showed that when lipoids were removed from the tubercle bacilli by prolonged extraction with acetone the bacilli became soluble in trypsin, and if animals were immunized with tryptic digests of acetone-extracted bacilli they produced a high yield of precipitins. Dreyer altered the method by a preliminary boiling in formalin after which the bacilli were extracted with acetone until they were no longer acid-fast and he introduced a vaccine (diaplyte) prepared in this way, but unfortunately it did not in practice fulfil the high expectations entertained on its introduction.

Immunization with living attenuated cultures. Many attempts have been made to obtain satisfactory immunity to tubercle by inoculation with human or bovine bacilli attenuated in various ways, or with avian, piscine, or other forms of tubercle bacilli. An account of these is given by Bandelier and Roepke (1913). Calmette and Guérin (1906) have advocated a vaccine (B.C.G.) of a living culture attenuated by growth in bile as a method of establishing immunity in the newly born offspring of tuberculous parents, and Calmette (1928) has published statistics claiming that very considerable immunity has been established. These statistics have been very severely criticized by Greenwood (1928), and it cannot yet be considered that Calmette has established his claim.

REFERENCES.

BANDELIER, B. E. G. & ROEPKE, O., 1913, *Tuberculin in diagnosis and treatment*, London.

CALMETTE, A., 1927, *Ann. Inst. Pasteur*, **41**, 201 ; 1928, *ibid.*, **42**, 1.

CALMETTE, A. & GUÉRIN, C., 1906, *Ann. Inst. Pasteur*, **20**, 609.

DOUGLAS, S. R., 1921, *Brit. J. Exp. Path.*, **2**, 175.

DREYER, G., 1923, *Brit. J. Exp. Path.*, **4**, 146.

GREENWOOD, M., 1928, *Brit. Med. J.*, i, 793.

KOCH, R., 1890, *Deuts. med. Wschr.*, **16**, 1029.

SMITH, MAYNARD, 1909, *Brit. Med. J.*, ii, 1046.

WRIGHT, A. E. & DOUGLAS, S. R., 1904, *Proc. Roy. Soc.*, **74**, 159.

WRIGHT, A. E., 1909, *Studies in Immunisation*, London (Constable & Co.).

Preparation and Standardization of Tuberculin.

BY C. C. OKELL.

Old tuberculin may be made from human or bovine strains, and, although it is customary to designate that derived from human strains

'T', and that from bovine strains 'PT', no satisfactory evidence has been produced to prove that there is any qualitative difference between the two preparations.

Preparation of old tuberculin (Koch, 1890).

A strain of human or bovine origin is chosen which is known to produce good tuberculin. The usual medium upon which the bacillus is grown is glycerin veal broth. This may be prepared by adding 2 per cent. peptone and 5 per cent. neutral glycerin to an extract of veal. The reaction is adjusted to pH 7·7. The bacillus should be grown as a surface film on the medium in large containers, which allow as much air space as possible. A young rapidly growing surface film should be used as the inoculum. The bottles are protected from shaking and incubated at 37° C. The tuberculin can be harvested about a month after the surface film across the medium is complete. The bottles are steamed for ½ to 2 hours according to their size, and their contents are strained through long-cloth, muslin or paper filters. The material is then bulked and evaporated to one-tenth of its original volume in shallow enamel pans over a boiling water bath. The process is assisted by an electric fan. The tuberculin may then be heated again to 100° C., and passed through a Berkefeld filter while still warm. Many minor modifications in the preparation are in use in different laboratories, and are reported on by Calmette and de Potter in their symposium on Tuberculin (1926). The unconcentrated filtrate is sometimes used and is named T.O.A. Its potency is about one-tenth of the same material concentrated, and there is no evidence that any active material is lost in concentration.

The other type of tuberculin which is still much used in medicine is bacillary emulsion (B.E.). Koch's (1901) method of preparing B.E. was to grind the living bacilli until they were killed and had lost their acid-fast properties. A well-grown culture from a solid medium or a film from a fluid medium is dried and ground in a revolving ball-mill until no intact acid-fast organisms are left in the preparation. The process of grinding may take several months. When grinding is complete the material is emulsified in saline and diluted so that the number of mgms. of solid matter in 1 c.cm. is suitable for human dosage. The final material is tested for sterility and for the presence of living tubercle bacilli by animal test.

Standardization.

Laboratory methods for standardizing tuberculin depend upon the quantitative study of the effects of the active principle on tuberculous guinea-pigs in comparison with a standard tuberculin. In the past the usual standard has been the standard tuberculin issued by the Staats-Institut für Experimentelle Therapie, Frankfurt. A number of guinea-pigs (20 to 50) of 300 to 350 gm. in weight are inoculated subcutaneously with 0·25 to 5 mgm. of a young growth of *B. tuberculosis.* If the inoculum is suitable the batch of guinea-pigs becomes appropriately sensitive to the intradermic and von Pirquet tests in about 3 weeks, and to the subcutaneous test in 6 to 8 weeks. For intradermic tests and von Pirquet tests it is preferable to use white or cream guinea-pigs.

Intradermic method (Römer and Joseph, 1909, 1910; Lewis and Aronson, 1923; Eagleton and Baxter, 1923). The reactions following the injection of 0·2 c.cm. of a series of dilutions of a tuberçulin are compared and matched with a similar series of dilutions of the standard tuberculin. The injections are made intradermally on the depilated skin of tuberculous guinea-pigs. The degree of sensitiveness of the guinea-pigs should be such that a 1/2,000 to 1/4,000 dilution gives a readable skin reaction. The usual range of dilutions put up is 1/500, 1/1,000, 1/2,000 and 1/4,000. Readings are best made 18 to 24 hours after injection. If the 1/500 dilution of the tuberculin of unknown potency gives a reaction of the same size as the 1/1,000 dilution of the standard tuberculin, the tuberculin is approximately half the strength of the standard tuberculin. The reactions due to the successive dilutions must be properly graded. The most potent dilution should give a well-marked reaction, which, however, should not be necrotic, and the least potent dilution of the series should show a much less marked reaction than the most potent. It is the comparison of reactions over the zone between the maximum reaction, and the reaction which is only just readable which gives the most accurate results. For practical purposes the results on at least six guinea-pigs should be collated.

Subcutaneous method (Koch, 1891; Otto and Hetsch, 1927). The batch of tuberculous guinea-pigs may be considered sufficiently sensitive for the test when 0·1 c.cm. of the standard tuberculin kills 3 out of 6 animals. The effect of a 0·1 c.cm. dose of the tuberculin under test should be compared with that of the same dose of the standard tuberculin. If the death-rates are not similar it is necessary to readjust the dose of the test tuberculin until similar rates (preferably about 50 per cent. mortality in each group) are produced. The strength of the tuberculin under test can thus be calculated. The previous use of the intradermic test effects a considerable saving of animals (Eagleton and Baxter, 1923). At least six guinea-pigs should be put on each of the tuberculins and this number allows of a rough comparison only (vide *infra* under error of test).

von Pirquet 'scratch' method. This method, which is of little value for quantitative purposes depends, like the intradermic method, on the comparison of reactions caused by the tuberculin under test with those caused by the standard material. The tuberculins are generally used undiluted and diluted 1/4, 1/8, 1/16 and 1/32. Three parallel scratches are made for each dilution and a drop of the dilution applied to each scratch. The readings are made 18 to 24 hours later.

Spermatocyte reaction of Long (Long, 1925). This depends upon changes produced in the testis of tuberculous guinea-pigs as a result of intratesticular injection of tuberculin. A greater constancy and reliability is claimed for it than for other guinea-pig methods. Hetsch, Schlossberger, Wichmann (1928) and also the present writer have not found the test comparable in quantitative accuracy with the intradermic or even the subcutaneous method in guinea-pigs.

Other methods. Methods of measurement based on the flocculation phenomenon described by Dreyer and Vollum (1924) are not applicable (Okell, *et al.*, 1926). It is obvious from the work of Mueller (1926), Laidlaw and Dudley (1925) and others that the material in tuberculin, which gives rise to the precipitation, is not the same substance as the active principle. The complement-fixation test (Watson and Heath, 1924), which appears to depend upon the same phenomenon as the flocculation test, has been found unsatisfactory as a measure of tuberculin by Calmette and de Potter (1926) and the present writer.

Tuberculins may be compared on tuberculous human beings and cattle, but few quantitative experiments have been recorded and little is known of the error of the methods. From the unpublished work of Dalling on cattle, and Cummins on human beings, in co-operation with the present writer and his colleagues, it seems reasonably certain that the error of any practicable test on human beings or cattle is considerably greater than in the intradermic test on guinea-pigs, but both cattle and human titrations are confirmatory of guinea-pig titrations. It may be taken that standardization by the intradermic test in guinea-pigs or by a sufficiently extended subcutaneous test is more than adequate for evaluating tuberculin either for human or bovine use.

The experimental error in standardizing old tuberculin.

The error of the various guinea-pig tests have been worked out by Okell and Parish (1927), who find that the intradermic test is more accurate on an ordinary laboratory test than the subcutaneous or von Pirquet methods, differences representing a 40 per cent. drop in potency being almost always detectable if about six guinea-pigs are used, and finer differences on a larger series of guinea-pigs. Calmette and de Potter (1926) also accept the intradermic method as the best. The test, however, calls for considerable experience. The subcutaneous test will also show differences of 40 to 50 per cent. if enough suitable guinea-pigs are used, but the cost in guinea-pigs of attaining a given degree of accuracy is much greater than with the intradermic test. An advantage of the subcutaneous method is that there is practically no personal factor in its interpretation.

The von Pirquet test on guinea-pigs may be able to detect a 50 per cent. drop in potency, if the tuberculins compared are strong, but in many cases the estimate may be very inaccurate, and it is possible to make such gross errors as to state that a given sample is 50 per cent. of standard strength when in reality it is tuberculin-free. For further information in connection with the accuracy of tuberculin tests, reference may be made to the paper by Okell and Parish (1927).

Potency of old tuberculin preparations.

Old tuberculin is not difficult to prepare when a suitable strain of the bacillus is employed, and a large number of samples examined at random are of approximately standard potency. Occasionally, however, samples are found which are considerably below Frankfurt standard value ; even a strain of bacillus which has consistently produced good tuberculin may fail without warning to do so. It is, therefore, hardly necessary to emphasize that all old tuberculins used in human and veterinary medicine should be at least roughly standardized. Up to the present the usual standard of comparison has been the tuberculin issued from Frankfurt, but recently a standard of very similar potency to the Frankfurt tuberculin has been issued by the Medical Research Council's Laboratories. Materials placed on the market should either be approximately equal to the standard, or if not, a statement should be made of their potency as a fraction of the standard.

Very rarely does any ordinary preparation exceed twice the potency of the Frankfurt standard. By the use of a medium containing potato extract and liver broth, S. R. Douglas (unpublished) made with one strain at least, tuberculin of 2 to 4 times the potency of standard. The writer has examined many dozen samples of old tuberculins prepared in laboratories in different parts of the world and has never met with any sample that has been more than twice standard strength with the exception of samples prepared by Douglas's method.

Stability and keeping properties of old tuberculin.

Undiluted old tuberculin is an extremely stable biological reagent and may be kept for many years at ordinary temperatures without loss in potency. It will even stand heating in the autoclave for half an hour at 15 lb. per square inch pressure without detectable change, and the deterioration is hardly appreciable after autoclaving for 2 hours at 10 lb. When diluted in saline it is less stable. A 1/1000 dilution kept at ordinary room temperatures had lost about 40 per cent. of its potency in 10 weeks and about 60 per cent. in 12 months. Sterile dilutions of old tuberculin may safely be used for one month after preparation even if kept at room temperature.

REFERENCES.

CALMETTE, A. & DE POTTER, F., 1926, *League of Nations Report*, C.H. 429, Geneva.
DREYER, G. & VOLLUM, R. L., 1924, *Lancet*, Lond., ii, 1003.
EAGLETON, A. J. & BAXTER, E. M., 1923, *Brit. J. Exp. Path.*, **4**, 289.
HETSCH, H., SCHLOSSBERGER, H. & WICHMANN, F., 1928, *Deuts. med. Wschr.*, **54**, 607.
KOCH, R., 1890, *Deuts. med. Wschr.*, **16**, 1029; 1891, *ibid.*, **17**, 101 & 1189; 1901, *ibid.*, **27**, 829.
LAIDLAW, P. P. & DUDLEY, H. W., 1925, *Brit. J. Exp. Path.*, **6**, 197.
LEWIS, P. A. & ARONSON, J. W., 1923, *Amer. Rev. Tuberc.*, **7**, 404.
LONG, E. R., 1925, *J. Infect. Dis.*, **37**, 368.
MUELLER, J. H., 1926, *J. Exp. Med.*, **43**, 1 & 9.
OKELL, C. C. & PARISH, H. J., 1927, *Brit. J. Exp. Path.*, **8**, 170.
OKELL, C. C., PARISH, H. J., O'BRIEN, R. A., DREYER, G., VOLLUM, R. L., CUMMINS, S. L. & BUXTON, J. B., 1926, *Lancet*, Lond., i, 433.
OTTO, R. & HETSCH, H., 1927, *Arb. Inst. exp. Ther. Frankfurt*, Heft 19.
RÖMER, P. H. & JOSEPH, K., 1909, *Beitr. Klin. Tuberk.*, **14**, 1 ; 1910, *ibid.*, **17**, 427.
WATSON, E. A. & HEATH, L. N., 1924, *J. Amer. Vet. Med. Ass.*, **66**, 24.

CHEMOTHERAPY IN TUBERCULOSIS.

BY S. L. CUMMINS.

Factors Unfavourable to Chemotherapy.

The chemotherapy of bacterial diseases in general has not proved an easy task, nor, up to the present, has it been very successful. In tuberculosis, chemotherapy encounters two special difficulties at the start; the devascularized character of the tuberculous foci on the one hand and the resistant nature of the tubercle bacillus on the other.

A good idea of the difficulty presented by the destruction of blood-vessels in tuberculous areas may be gained from the observations of Secher and Thisted (1927), who, by injections of 10 per cent. cinnabar gelatin into tuberculous lungs and subsequent radiological examination obtained macroscopic evidence of the vascular poverty of the central parts of the lesions, while histological examinations of sections after injection of 10 per cent. China ink afforded microscopic confirmation.

The power with which the tubercle bacillus is endowed to resist the penetration of its cell-substance by chemicals is well known. A germ endowed with such resistant qualities would be difficult to kill by chemical agents even if free in the blood-stream; but in tuberculosis it is not with a mere septicæmia that the chemotherapeutist has to deal, but with a pathological complex consisting of a bacillary colony with its surrounding tuberculous tissue. This unit, the tuberculous focus, is by no means uniform in character. Even in the same host, some foci may be undergoing a slow process of spontaneous cure accompanied by central calcification and peripheral fibrosis, others may be increasing in size by the formation of daughter tubercles, through the fusion of which large caseous areas may subsequently be formed. Other foci again may be stationary, as if a rough balance had been struck between the multiplication of bacilli and the tension of bacillary products within the caseous tubercle on the one hand, and the penetrability of the cellular barrier separating them from the blood in the peripheral capillaries on the other; a balance liable to be upset by anything leading to hyperæmia of the focus. Jobling and Petersen (1914) find, in the formation of soaps from the fatty acids derived from the tubercle bacilli, and the anti-enzyme action of these soaps, a possible explanation of the persistence of these caseous foci in the organs and tissues. Whether this be the true explanation or not, such caseous areas constitute a barrier zone between the circulating blood and the tubercle bacilli.

Penetrability of Tuberculous Foci by Substances in Solution.

The fact, however, that tubercles readily undergo calcification by deposit of lime salts brought to their peripheral boundary by the blood-stream is, in itself, a proof that tuberculous foci may be penetrated by substances in solution. This was further established by Lewis (1912) with isamin blue and trypan red, and by Evans, Bowman and Winternitz

(1914), who studied the histogenesis of tubercles in rabbits by vital staining with trypan blue. De Witt (1923) who laid stress on this permeability of tubercles by chemicals in solution, quotes the work of Wells and Hedenburg to the effect that injected iodide of potassium enters freely into tubercles. She points out that, while normal cells, with the exception of renal cells, are not perfectly permeable to iodides, they lose their impermeability when killed or injured. A barrier of cells, killed in the process of tuberculous caseation, might thus become easily permeable for crystalloids present in the surrounding fluids.

Experiments with dyes. Ideas of this order greatly influenced the work of De Witt in her experiments on the chemotherapy of tuberculosis. Thus, in 1913[2], she worked with methylene blue on lines similar to those adopted earlier by von Linden, whom she quotes. She showed, in guinea-pigs, that methylene blue penetrated the tuberculous focus and even stained the tubercle bacilli within it, but found that it could not be depended upon as a bactericidal agent. This attempt to exploit the penetrative power of antiseptic dyes for the experimental treatment of tuberculosis proved disappointing in itself, but led on to a further series of efforts in which it was sought to *carry* less penetrative, but more actively bactericidal substances into the interior of tubercles in chemical combination with diffusible dyes. Ideas of this order appear to underlie many of the attempts of De Witt (1921) to find mercury compounds suitable for chemotherapy in tuberculosis.

Bactericidal and Bacterio-inhibitory Substances.

While the work already quoted had established the possibility of reaching tubercle bacilli in the interior of tuberculous areas by means of chemical substances introduced into the blood-stream, other investigators had been concentrating their efforts upon the discovery of bactericidal agents capable of destroying or hindering the growth of the tubercle bacillus itself.

R. Koch (1890[2]) had already investigated the bactericidal and culture-inhibiting action of many substances and had shown that gold cyanide was able completely to inhibit the growth of tubercle bacilli even in a dilution of 1 in 2 millions, *in vitro,* though finding, however, that it was of no use in treatment *in vivo.*

Chaulmoogra oil and sodium morrhuate.

A more hopeful line of work appeared to be opening up when, following the successful use of chaulmoogra oil derivatives in leprosy by Rogers, Hollman and others, Walker and Sweeney (1920) found that the fatty acids of the chaulmoogra series possessed specific bactericidal activity for several types of acid-fast bacilli, being, for these organisms, a hundred times more lethal than carbolic acid. Similar findings were recorded by Lindenberg and Pestana (1921) who reported, amongst other things, that the fatty acid of *Taraktogenus Kurzii* prevented the growth of avian tubercle bacilli in a dilution of 1 in 100,000. Kolmer, Davis and Jager

(1921), however, tried out the oil expressed from the seeds of *Taraktogenus Kurzii* on tubercle bacilli both *in vitro* and *in vivo* in guinea-pigs, and found that it was without effect. Sodium morrhuate, suggested by Rogers (1919) for clinical trial in tuberculosis, and found by Shaw-Mackenzie (1921) to exert a marked accelerating action on lipase, was tested by the Medical Staff of the Welsh National Memorial Association on upwards of 200 patients, and was reported by Cummins (1925), who analysed their clinical records, to be without demonstrable effect in treatment. Something, however, may yet be accomplished in the treatment of tuberculosis by derivatives of the unsaturated fatty acids of chaulmoogra and other oils. The success of these preparations in leprosy gives ground for hope. So far, little has been demonstrated beyond their bactericidal effects *in vitro*. Extended experiments in their application to the tuberculosis of animals are required before further progress in human therapy can be expected.

A summary of metallic therapy.

The rare earth metals and others. Cerium and some others of the rare earth metals were found by Frouin (1912) to stop all growth of the tubercle bacillus *in vitro*, and success has since been claimed for these metals in treatment. Walbum and Mørch (1923) had shown that manganese, cadmium and other metals had a marked effect in increasing the titre of antitoxic sera, and Walbum (1927) later reported very significant results in the treatment of experimental tuberculosis of rabbits and mice. Helms and Frederiksen (1927) have observed very encouraging results with Walbum's method in the treatment of patients, and it is likely that manganese and cadmium therapy will deserve close attention in future work on tuberculosis. The work of De Witt (1921) with mercury compounds has already been referred to and has been particularly thorough. Some of her preparations have given very suggestive results in guinea-pigs. Arsenical compounds, tested by Arkin and Corper (1916), were without effect.

Copper. The Gräfin von Linden (1912), in a series of investigations communicated between 1912 and 1921, tested out a series of copper compounds and claimed for some of them definite powers to inhibit or destroy the tubercle bacillus ; growth was stopped by dilutions so high as one in a million. Her co-worker, Meissin (1914), who reported fully on the toxicity of copper salts, also obtained encouraging results in treatment. Following the lead of von Linden, a Japanese worker, Koga (1916), prepared a compound of copper cyanourate with potassium cyanide, called ' Cyanocuprol ', with which he believed that good results were obtained in 63 patients treated.

Gold. The observation of R. Koch, already referred to, of the marked bactericidal power of gold cyanide for the tubercle bacillus led later workers to devote closer attention to gold therapy. Bruck and Glück (1913) used gold potassium cyanide in the treatment of lupus, the compound being given by intravenous inoculation, with very favourable results in some cases. Arthur Mayer (1914) tried a combination of gold potassium cyanide and borcholin, a cleavage product of choline, first in guinea-pigs and rabbits, then in human patients, apparently with some success. Then came an important series of communications by Spiess and Feldt (1915) on monocantharidyl-ethylenediamine-aurous-cyanide or 'Aurocantan' and, later, a series of papers by Feldt (1917) on P-amino-o-aurophenolcarbolic acid or 'Krysolgan', which brought the question of gold therapy to the attention of the medical profession, especially in Germany.

Perhaps owing to the fact that most of these highly interesting communications were published during the war, the work of Spiess and Feldt appears to have attracted but little attention in England. A complete account of the use of Krysolgan in tuberculosis and leprosy, published by Feldt (1923), should be consulted for bibliography and detailed results.

A year later, Holgar Møllgaard (1924) produced, from the Royal Veterinary and Agricultural College of Copenhagen, his work on sodium aurothiosulphate or 'Sanocrysin', thus advancing the chemotherapy of tuberculosis by gold compounds in a form so challenging as to excite a world-wide interest in the problem.

Similarities in effects of metallic compounds.

On the chemical treatment of tuberculosis up to 1923, excellent summaries exist by Hans Schlossberger (1923) and by Lydia De Witt (1923). It appears advisable, therefore, to concentrate upon the work of Møllgaard, which, appearing in 1924 after the publication of the two articles above quoted, still requires a more detailed review. This course is convenient, also, because of the fact that the problems raised by the work of von Linden with copper salts, by Spiess and Feldt with aurocantan and krysolgan, and by Walbum with manganese and cadmium, are, in many respects, identical with those raised by Møllgaard with sanocrysin. A consideration of the latter compound must, therefore, involve many references to other work on metallic preparations. Schlossberger, in his closing paragraph, summed up the position of chemotheraphy in 1923 in the following words: 'The number of substances tested for their therapeutic activity in experimental and clinical tuberculosis is, as the foregoing summary shows, very great. But systematic investigations after the manner of Ehrlich in his chemotherapeutic studies of the protozoal diseases, involving complete researches into whole groups of chemical compounds and their activity in relation to tuberculosis have been very few up to the present time'.

Shortly after these words were written, appeared the work of Møllgaard, of which it may be said that, whatever the final verdict as to the mode of action of sanocrysin, it represents a serious attempt to apply the methods and principles of Ehrlich to the treatment of tuberculosis. Before, however, attempting to discuss this new development, it is well to return for a moment to the effects of gold and copper salts upon the tuberculous organism as observed by earlier workers.

von Linden (1920), who believed her copper compounds to be bactericidal in Ehrlich's sense, found strong support for her opinion in the responses of tuberculous persons to the inoculations: 'Allgemein- und Herd-reaktionen die bei Lungentuberkulose beobachtet werden und der Tuberkulin-reaktion analog verlaufen'. Feldt (1924) noted similar reactions with krysolgan and laid special emphasis on the different behaviour of tuberculous and non-tuberculous persons after inoculation. He reports that Spiess, in carcinoma patients inoculated with krysolgan in the course of experiments in the production of secondary rays in Roentgenotherapy, found that doses up to 0·5 gm. were usually well tolerated by these non-tuberculous persons; while, on the other hand, Feldt observed that patients with the exudative type of phthisis often gave both focal and general reactions even to a dose of 0·0001 gm. This undoubtedly specific action of krysolgan was thought by Feldt to depend on a *catalytic* action of the gold salt in speeding up the slow spontaneous autolytic processes normally at work in tuberculous lesions, and the secondary liberation of the specific antibodies and enzymes from these foci. He draws the closest parallel between the reactions caused by krysolgan and by tuberculin, but finds the effects of the former to be more local, due in part to the rapid conversion of the liberated toxins into non-toxic bodies, and in part to the growth-limiting effects of krysolgan on the tubercle bacilli, 'an effect which is admittedly impossible for tuberculin'.

Thus, while von Linden explained the tuberculin-like reactions by the destruction of tubercle bacilli, Feldt, and later Walbum, attributed them to catalytic action in the tuberculous focus. It will be perceived that the *rationale* of experiments in the chemotherapy of tuberculosis by salts of metals depends upon the view held as to the cause of these 'specific' reactions.

Sanocrysin as a Type of Heavy Metal Compound.

Characters of sanocrysin.

Møllgaard, in his search for a substance active against tuberculosis, laid down in advance certain characters which it must possess in order to fulfil this purpose. Such a substance must, he says, be soluble in water, rapidly diffusible, a real complex without reactions on gold ions. It must be only slowly decomposible in the organism and its decomposition there must take place without the liberation of gold ions or other poisonous bodies. After testing and discarding many chemical combinations, he turned his attention to the compounds of the heavy metals with thiosulphuric acid. Fordos and Gélis, quoted by Møllgaard, had produced a compound which they considered as probably a double thiosulphate of gold and sodium. A close study of the substance gave Møllgaard the idea of 'a synthesis quite different from the method of Fordos and Gélis' by which the compound 'is easily built in pure form containing, after one crystallation, 90 to 95 per cent. of the gold employed at the synthesis'. The compound so produced may, he thinks, be written as $\left[\mathrm{S_2O_3Au}\right]\begin{matrix}\mathrm{Na}\\ \mathrm{Na_2S_2O_3}.\end{matrix}$ This compound he has registered under the name of 'Sanocrysin', and he believes it to possess, to a great extent, 'the physico-chemical properties which we must demand of a chemotherapeutical compound of a heavy metal'. It is a snow-white substance crystallizing in long needles, readily soluble in water, its aqueous solutions being nearly neutral. The negatively charged ion, $\mathrm{AuS_2O_3}$, is rapidly diffusible through a membrane.

Action on tubercle bacilli in vitro.

Møllgaard studied the growth-inhibiting and bactericidal powers of this compound by testing it in concentrations of from 1/1,000 to 1/10,000,000 of nutrient bouillon. After 4 weeks' incubation at 37° C. it was found that growth of tubercle bacilli had been completely prevented by a concentration of 1/100,000 of sanocrysin and markedly retarded by a concentration of 1/1,000,000. These observations were confirmed by Sweany and Wasick (1925), but O. Bang (1926) and Calmette, Boquet and Nègre (1926) found growth inhibition to be much less marked, being usually lost in dilutions greater than 1/5,000.

Madsen and Mørch (1926) have done much to explain these differences by showing that the action of sanocrysin varies with the strain of tubercle bacillus employed, and the composition of the culture medium used for the test. These factors explain, perhaps, the findings of R. M. Fry (1926), who, using Wright's 'blood-clot' technique, found that sanocrysin had but little action on tubercle bacilli.

Action on tubercle bacilli in vivo.

While recognizing the importance of these '*in vitro*' tests, Møllgaard had stated from the first that the real decision as to the value of sanocrysin must turn on its effects on 'the experimentally induced or spontaneously contracted tuberculosis of animals'. Before embarking on these tests, it was necessary to establish clear data as to its effects on the non-tuberculous organism.

Action on normal animals. As was to be expected, sanocrysin was found to give rise to toxic effects when given in large doses to healthy animals. In the words of Møllgaard, 'the principal features of this intoxication are the same as generally found in intoxications with the heavy metals; ascending paralysis, nephritis, œdema of the pia. The animals die of respiratory paralysis in a somnolent condition'. The first necessity, then, was to establish the *dosis tolerata* for the animals to be used in therapeutic experiments. Møllgaard found that it increases from the lower to the higher mammals. He tabulates his results as follows, per kgm. of live weight:

Mice, subcutaneous	12 cgm.
Guinea-pigs, intraperitoneal	3–4 „
Rabbits, subcutaneous	8 „

Rabbits, intravenous	4 cgm.
Calves, intravenous	6 „
Goats, intravenous	3 „
Macacus sinicus, intraperitoneal	6 „

Within these limits, he found that sanocrysin could be given to healthy animals without serious intoxication, and, when administered in moderate doses, without any disagreeable results. A 4·5 per cent. solution in distilled water is practically isotonic with blood and is suitable for intravenous use, while subcutaneous and intramuscular injections cause no more than a transient local œdema. The circulation, respiration and body temperature are not affected, and an intravenous dose of 1 cgm. per kgm. of live weight is without visible effect on the kidneys. In rabbits, a dose so large as 2 cgm. per kgm. intravenously proved to have no serious influence upon the renal efficiency ; while the liver, too, seemed to escape without injury, no icterus being observed even after comparatively large doses. Møllgaard noted that the gold was to be found in considerable quantity in the urine for from 4 to 6 days after an intravenous inoculation. This slow excretion he regarded as important, since, in treatment, it might ' augment the possibilities of the substance to diffuse into the tuberculous tissue '.

McClusky and Eichelberger (1925), working with doses of 2 cgm. and upwards, per kgm., in normal dogs, found that the intravenous injection of sanocrysin caused diarrhœa, vomiting and albuminuria, the amount of albumin in the urine being in proportion to the weight of gold salt injected. They found, further, that about 60 per cent. is excreted through the kidneys, the greater part within the first six days, though smaller quantities continue to appear in the urine up to about 30 days ; from 7 to 18 per cent. are excreted by healthy dogs *after* the sixth day. While their observations were made with rather large doses and do not run counter to the general conclusion of Møllgaard that sanocrysin may be injected into the healthy organism with impunity in moderate doses, McClusky and Eichelberger show clearly that sanocrysin gives rise to gold poisoning in doses well within the limits of the so-called *dosis tolerata*, and that the effects produced are similar to some of the effects noted after ' therapeutic doses ' in tuberculous patients. They show, too, that the excretion of gold in the urine continues for much longer than Møllgaard had, at first, believed.

Action on tuberculous animals. While, however, doses of from 1 to 2 cgm. per kgm. of live weight are tolerated without trouble by most laboratory animals, sanocrysin in these doses gives rise to very serious symptoms when administered to animals suffering from experimental tuberculosis. It will be recalled that R. Koch (1890^{2}) noted a marked contrast in the behaviour of the healthy and the tuberculous organism to tuberculin. He found that, while healthy guinea-pigs could tolerate up to 2 c.cm. without disturbance, a dose of 0·5 c.cm. led to the rapid death of a guinea-pig infected four or five weeks previously with a virulent strain of tubercle bacillus.

Møllgaard, after numerous experiments, came to the conclusion ' that the injection of sanocrysin, in doses which are not poisonous in themselves, kills the tuberculous guinea-pig by producing a tuberculin shock '. A close study of this ' shock ', produced in artificially tuberculized calves by the intravenous inoculation of sanocrysin, showed that the condition was manifest in three stages: First there is albuminuria, more or less grave according to the severity of the tuberculous infection. Next appears a toxic myocarditis with indistinct heart-sounds, arhythmia and a slow pulse. Thirdly comes the onset of œdema of the lungs and cyanosis; and, finally, collapse and death with a flow of foaming fluid from the mouth and nose. Coincidently with the lung œdema, there is a rapid fall of the temperature to below normal.

For Møllgaard, this ' shock ' meant that a considerable number of tubercle bacilli had been killed by the sanocrysin and their endotoxins liberated into the general circulation. He quoted the observations of Prudden and Hodenpyl, of Strauss and Gamaleia, of Calmette and Breton, Kelman, Engelhardt and others as to the highly toxic properties of dead tubercle bacilli for the tuberculous organism and concluded ' that the introduction of any chemical compound which kills tubercle bacilli into a seriously infected tuberculous organism must produce a very serious intoxication unless something can be done to neutralize the liberated toxins '.

Antituberculous serum. With this view as to the causation of ' shock ', it was a natural next step to attempt to neutralize it by means of an anti-tuberculous serum. Møllgaard's experiments in this direction are very striking and they are supported by the clinical finding of K. Secher (1926) and others, by whom it has been found that a timely use of the serum can cut out the ' shock ' and ' albuminuria ' effects of sanocrysin. His theory is also supported by the fact that ' animals with a *more chronic tuberculosis* often show a high immunity ' to the effects of sanocrysin, tolerating relatively large doses without shock or albuminuria.

In animals spontaneously infected with and relatively ' immune ' to tuberculosis, the response to sanocrysin is a *rise* of temperature instead of the ' drop ' characteristic of shock; and it is to be noted, in this connection, that the administration of the serum to ' shock ' cases converts the temperature fall to a rise. It is significant, too, that, during a course of sanocrysin inoculations, while the earlier doses are often followed by ' reactions ' the later ones are well tolerated, suggesting that the organism has been ' desensitized '.

Confirmatory and other experiments.

Møllgaard's animal experiments have been criticized by Dixon (1925), Bang (1926) and others, on the ground that he produced his experimental tuberculosis with strains of bacilli of relatively low virulence. While this objection hardly explains why animals untreated by the serum died of shock and those treated recovered, it does raise a point of great importance in connection with all investigations in the therapy of experimental tuberculosis. It is obviously impossible to obtain, in animals infected with virulent strains, a tuberculosis clinically comparable with that of human adults, while the use of strains of lower virulence lays the worker open to the criticism that his 'favourable' results may have resulted from spontaneous cure. This question is well discussed by Gorke and Tõppich (1922) in connection with their krysolgan experiments.

Treatment of experimental tuberculosis. In connection with sanocrysin, the results of those who have since worked with virulent strains have been conflicting. Madsen and Mørch (1926) have confirmed Møllgaard's work in large numbers of trials with rabbits. Although they found that sanocrysin treatment, started on the same day as the infection, was useless, they were able to arrest or cure the disease in nearly all cases when the start of treatment was deferred to the fourth day after the production of the infection. On the other hand, O. Bang (1926), Calmette, Boquet and Nègre and others were unable to obtain any results at all with sanocrysin in the treatment of infected calves, rabbits and guinea-pigs.

Using a technique designed to produce, if possible, a closer approximation to human tuberculosis, by the employment of larger doses of less virulent tubercle bacilli in rabbits, Cummins (1926) obtained strikingly successful results with sanocrysin, relying on the use of 'control' animals to prove that the infections were sufficiently severe to produce disease and death in those left untreated.

In view of the finding of Madsen and Mørch that, in order to be effective, sanocrysin treatment must not be started before the fourth day after the infection, Bang (1927) repeated his former work on these lines, but again without favourable results. Madsen and Mørch (1928) reported further success and finally took part in a combined research which has since been carried out, Bang, Madsen and Mørch (1928) repeating their experiments on a comprehensive scale, but, on this occasion, with completely negative results. It appears, however, that the experiment was not a good one, many animals being lost through an intercurrent *Pasteurella* infection, and the type of tuberculosis differing from that produced previously by infecting doses believed to be identical. That these doses, while representing the same weight of the same strain, may have been very different in 'virulence' is suggested by recent observations on 'microbic dissociation' of tubercle bacilli by Petroff (1927), who shows that, by appropriate methods, tubercle cultures may produce 'rough' and 'smooth' types of colony with markedly different virulence. It is clear that, if Petroff's observations are confirmed, this factor will have to be taken into account in future experiments in the chemotherapy of tuberculosis. Møllgaard (1928) has recently produced further work which confirms and amplifies his previous findings, but which has also led him to modify somewhat his original view of a direct action of the unaltered sanocrysin upon the parasite; but he still regards the 'shock' and 'temperature' reactions as due to the liberation of toxins, not to any poisonous action of gold.

Treatment of human tuberculosis. When looked at from the biological point of view, the results of sanocrysin treatment in human tuberculosis afford a large measure of confirmation of Møllgaard's original findings in animals, though not necessarily of his interpretations of those findings. The very 'failures' which have led certain clinicians to condemn sanocrysin have depended in many cases upon the occurrence of those symptoms of 'shock', 'albuminuria' and low temperature which Møllgaard observed in calves, and cited as a proof of the liberation of tuberculous 'toxins' through the destruction of a large number of tubercle bacilli. The 'successes', too, have supported his laboratory findings, the sputum becoming free from bacilli, the physical signs becoming less marked and the patient returning, at least for the time being, to normal weight and good health.

Mode of action.

It is not proposed, in this summary, to deal with the clinical records, which belong less to the bacteriological than to the general aspect of medical science. The problems which attract the attention of those interested in chemotherapeutical research are those concerned with the mode of action of salts of the heavy metals in tuberculosis, and these problems, thanks to the extensive animal experiments carried out by Møllgaard and others, can conveniently be discussed in connection with sanocrysin.

It would seem to be established that the 'reactions' of tuberculous persons and animals to the gold salts, sanocrysin, krysolgan and aurocantan and to the copper compounds, lecutyl and cyanocuprol, are, as maintained by Møllgaard, Feldt and von Linden, analogous with the tuberculin reaction. R. Koch (1890[2]) himself described the reaction of a tuberculous subject to a dose of 0·01 c.cm. of tuberculin, which was well tolerated by a healthy person, as accompanied by evanescent jaundice and measles-like eruptions on the chest and shoulders, ushered in by shivering, increased cough, high temperature and perhaps vomiting as well as lassitude and limb pains. Diarrhœa and transient albuminuria have been noted by other observers. Those who have used sanocrysin in treatment will recognize these symptoms as precisely similar to the sequelæ of fairly large doses of the gold salt in human cases. It seems probable that some of these symptoms may be produced in healthy subjects by sufficiently large doses of the heavy metals, and there must, therefore, remain some uncertainty, in individual cases, as to whether 'tuberculin reaction' or 'gold poisoning' is responsible for some of the disagreeable sequelæ of sanocrysin. Probably the early albuminuria, the measles-like rashes and early icterus are due to the 'tuberculin reaction', while the late albuminuria, the diarrhœa, the glossitis and the late desquamative dermatitis sometimes met with are due to 'metallic poisoning'. But as to two of the symptoms produced by sanocrysin in the tuberculous organism there can be but little doubt. The 'shock' and temperature appear to be the result of a 'tuberculin reaction' evoked by the inoculation of a gold salt into the tuberculous organism.

These reactions are almost certainly ‘ specific ’, and due to a direct action of the gold salt on the tuberculous foci. This is borne out by the fact that the reactions are, for a given dose, more severe the greater the extent of the disease ; and, for a given degree of infection, that the reactions are greater with larger doses of sanocrysin. Further, in tuberculous animals dying after sanocrysin, the foci of infection are seen to be intensely congested, proving that they have been directly influenced by the inoculation. Eichelberger and McClusky (1926) have shown that the percentage of gold recovered from the urine of tuberculous dogs inoculated with sanocrysin is about 10 per cent. less than the average recovered from non-tuberculous dogs ; and somewhat similar findings have been recorded by Frandsen (1925, 1926). This suggests that some, at least, of the gold is retained in or around the tuberculous foci. Gallinal (1927), by a microchemical technique, was able to see, in sections, that the gold was particularly visible in the tuberculous as opposed to non-tuberculous organs, though he noted that it lay, not *in* the tubercles, but in the tissues in the immediate vicinity of the tuberculous foci.

While it seems certain that sanocrysin, and the other heavy metal preparations causing ‘ specific ’ reactions, have a direct influence on the tuberculous foci, it is doubtful whether they have a direct bactericidal action on the parasite itself. Laboratory experiments have proved conclusively that sanocrysin does not sterilize emulsions of tubercle bacilli even after several days contact. It would seem as if Møllgaard’s original contention that the ‘ tuberculin reaction ’ following sanocrysin is caused by toxins liberated through the destruction of tubercle bacilli may have to be abandoned. Nor is any such theory necessary to explain the facts. It seems likely, from the effects of tuberculin itself, that there must be sufficient toxic material dissolved or adsorbed in the caseous areas to produce all the reaction phenomena observed after sanocrysin. All that appears to be required is the disadjustment of a balance, the upsetting of a state of tension, through hyperæmia of the capillaries in the periphery of tuberculous foci.

Whether this hyperæmia is brought about through a catalytic action of the gold salt, as believed by Feldt, or by a specific effect of the gold salt on the capillaries of tuberculous granulation tissue, or in some other way, the facts remain that sanocrysin does produce this hyperæmia and that this ‘ focal reaction ’ is associated with or causes a general reaction as well. That such reactions, if not excessive, often lead on to amelioration or cure, cannot be denied. How far this favourable effect depends on an *ictus immunizatorius* and how far on an inhibition of multiplication of the bacilli it is not yet possible to say. Possibly both factors play a part. In this connection the words of Robert Koch should not be forgotten : ‘ Es ist nicht nötig, wie irrigerweise noch vielfach angenommen wird, dass die Bakterien im Körper getötet werden mussen ; sondern es genügt, ihr Wachstum, ihre Vermehrung zu verhindern, um sie für den Körper unschädlich zu machen ’.

References.

Arkin, A. & Corper, H. J., 1916, *J. Infect. Dis.*, **18**, 335.

Bang, O., 1926, *Z. Tuberk.*, **44**, 298 ; 1927, *ibid.*, **47**, 286.

Bang, O., Madsen, T. & Mørch, J. R., 1928, *Acta tuberc. Scand.*, **4**, 39.

Brück, C. & Glück, A., 1913, *Münch. med. Wschr.*, **60**, 57.

Calmette, A., Boquet, L. & Nègre, L., 1926, *Rev. Tuberc.*, Paris, **7**, 169.

Cummins, S. L., 1925, *Ann. Report Welsh Nat. Memorial Assoc.*, p. 40 ; 1926, *Brit. J. Exp. Path.*, **7**, 47.

De Witt, Lydia, 1913[1], *J. Infect. Dis.*, **12**, 68 ; 1913[2], *ibid.*, **13**, 378 ; 1921, *ibid.*, **28**, 150 ; 1923, *The Chemistry of Tuberculosis* (Wells, H. G., De Witt, L. & Long, E. R., Williams & Wilkins Coy., Baltimore, U.S.A.), p. 351.

Dixon, W. E., 1925, *Tubercle*, Lond., **7**, 1.

Eichelberger, L. & McClusky, K. L., 1926, *Amer. Rev. Tuberc.*, **14**, 533.

Evans, H. M., Bowman, F. B. & Winternitz, M. C., 1914, *J. Exp. Med.*, **19**, 283.

Feldt, A., 1917, *Berl. klin. Wschr.*, **54**, 1111 ; 1923, *Klinische Erfahrungen mit Krysolgan*, Halle (Marhold) ; 1924, *Beitr. Klin. Tuberk.*, **57**, 269.

Frandsen, J., 1925, *Acta tuberc. Scand.*, **1**, 215 ; 1926, *ibid.*, **2**, 183.

Frouin, A., 1912, *C.R. Soc. Biol.*, Paris, **72**, 1034.

Fry, R M., 1926, *Brit. J. Exp. Path.*, **7**, 174.

Gallinal, J., 1927, *Z. Tuberk.*, **48**, 433.

Gorke, H. & Töppich, G., 1922, *Beitr. Klin. Tuberk.*, **53**, 132.

Helms, O. & Frederiksen, J., 1927, *Z. Tuberk.*, **49**, 18.

Jobling, J. W. & Petersen, W., 1914, *J. Exp. Med.*, **19**, 395.

Koch, R., 1890[1], *Deuts. med. Wschr.*, **16**, 756 ; 1890[2], *Gesammelte Werke*, J. Schwalbe, 1912, Leipzig (Georg Thieme), 661.

Koga, 1916, *J. Exp. Med.*, **24**, 107.

Kolmer, J. A., Davis, L. C. & Jager, R., 1921, *J. Infect. Dis.*, **28**, 265.

Lewis, P. A., 1912, *Arch. Intern. Med.*, **10**, 68.

Linden, Gräfin von, 1912, *Münch. med. Wschr.*, **59**, 2560 ; 1920, *Beitr. Klin. Tuberk.*, **44**, 117.

Lindenberg, A. & Pestana, B. P., 1921, *Z. ImmunForsch.*, **32**, 66.

Madsen, Th. & Mørch, J. R., 1926, *Acta tuberc. Scand.*, **2**, 99 ; 1928, *ibid.*, **4**, 12.

Mayer, A., 1914, *Beitr. Klin. Tuberk.*, **32**, 211.

McClusky, K. L. & Eichelberger, L., 1925, *Amer. Rev. Tuberc.*, **12**, 329.

Meissen, E., 1914, *Z. Tuberk.*, **21**, 409.

Møllgaard, H., 1924, *Chemotherapy of Tuberculosis*, Copenhagen ; 1928, *Acta tuberc. Scand.*, **3**, 337.

Petroff, S. A., 1927, *Proc. Soc. Exp. Biol.*, N.Y., **24**, 632.

Rogers, L., 1919, *Brit. Med. J.*, i, 147.

Schlossberger, H., 1923, *Handbuch der Tuberkulose*, **2**, 337, Leipzig (Brauer, L., Schroder, G., u. Blumenfeld, F.).

Secher, K., 1926, *Treatment of Tuberculosis with Sanocrysin and Serum* (Levin and Munksgaard), Copenhagen.

Secher, K. & Thisted, A., 1927, *Acta tuberc. Scand.*, **2**, 325.

Shaw-Mackenzie, J. A., 1921, *J. Trop. Med.*, **24**, 161.

Spiess, G. & Feldt, A., 1915, *Beitr. Klin. Tuberk.*, **52**, 365.

Sweany, H. C. & Wasick, M. M., 1925, *Amer. Rev. Tuberc.*, **12**, 316.

Walbum, L. E., 1927, *Z. Tuberk.*, **48**, 193.

Walbum, L. E. & Mørch, J. R., 1923, *Ann. Inst. Pasteur*, **37**, 396.

Walker, E. L. & Sweeney, M. A., 1920, *J. Infect. Dis.*, **26**, 238.

Administrative Measures

By S. R. Gloyne.

The administrative measures applied to the prevention of tuberculosis in Great Britain are summarized by F. J. H. Coutts (1924) and by Robertson and Porter (1928). These measures have resulted in process of evolution from the work of the Departmental Committee on Tuberculosis appointed by the Local Government Board in 1911. The recommendations of this committee are embodied in various Acts.

From the bacteriological side the following points in these regulations may be emphasized:

(1) *Bacteriological diagnosis.* Examination of sputum is provided for by the Local Authorities, generally at the Tuberculosis Dispensaries or Public Laboratories. The examination of tissues, or of excretions such as urine or fæces, requiring special laboratory equipment is not, as a rule, carried out at the dispensaries, and most Local Authorities make special arrangements with public laboratories. A similar procedure is adopted with immunological tests such as complement fixation.

(2) *Treatment.* Institutional treatment should not be confined to the early case which treatment may benefit. The advanced and heavily infected case which treatment is not likely to benefit needs institutional accommodation also in order not to be an infective focus in the home. For the rest the bacteriologist's usefulness in treatment is confined to assisting the clinician in (*a*) the use of tuberculin and vaccine therapy, (*b*) the testing of new immunological and chemotherapeutic remedies, and (*c*) providing bacteriological control of treatment by means of repeated examination of sputum, urine, blood, &c.

(3) *Prophylaxis.* The use of various types of antituberculosis vaccine as a means of prophylaxis is still in the experimental stage. From the nature of the case a long period of observation on large numbers of patients will be necessary before sufficient data can be obtained on which to base an opinion.

(4) The relation of the dairy industry and of tuberculous foodstuffs to tuberculosis has been discussed in Vol. III.

REFERENCES.

COUTTS, F. J. H., 1924, *Anti-Tuberculosis Measures in England*, Pamphlet prepared for the Society of Medical Officers of Health (England).

ROBERTSON, W. & PORTER, C., 1928, *Sanitary Law and Practice* (London).

CHAPTER V. TUBERCULOSIS IN COLD-BLOODED ANIMALS.

BY A. STANLEY GRIFFITH (UNIVERSITY OF CAMBRIDGE).

SPONTANEOUS lesions containing bacilli with the staining reactions of the tubercle bacillus have been found in a great variety of species of cold-blooded animals.

The causal relationship of the acid-fast bacilli with the lesions has been questioned by some writers, since, as Weber and Taute (1905) and Petrow (1907) pointed out, similar organisms may be found in the tissues of apparently healthy cold-blooded animals, as well as in the mud and slime in which they had lived. There is, however, little doubt that the bacilli obtained from the spontaneous lesions were the cause of the condition, at least in the majority of instances, since cultures are pathogenic for many species of cold-blooded animals, which is not the case with ordinary saprophytic acid-fast bacteria. On account of the superficial resemblance of the lesions to those caused in warm-blooded animals by the tubercle bacillus and the constant presence in them of abundant acid-fast bacilli, these conditions may properly be described as tuberculosis, though typical giant-cells are not associated with them.

Among cold-blooded animals living free tuberculosis is extremely rare. Küster (1913) examined 200 frogs and 50 other cold-blooded animals and found tuberculosis in the liver of 3 frogs only. In addition he found acid-fast bacilli abundantly present in the liver of another frog without macroscopic lesions. The author has carried out experiments with nearly 100 toads and examined many frogs and toads which had never been in captivity and failed to find spontaneous lesions or acid-fast bacilli.

The great majority of the recorded instances of natural tuberculosis have occurred in animals which had been for some time kept in captivity in aquariums or zoological gardens. The species of animals in which lesions associated with the presence of acid-fast bacilli have been found are fresh- and sea-water fish, frogs, turtles, snakes and alligators.

Fish.

This order is placed first because the first culture of acid-fast bacilli from cold-blooded animals was obtained from a fish.

Fresh-water fish. Bataillon, Dubard and Terre (1897) discovered in a carp from a fish hatchery a carcinoma-like tumour the size of a pigeon's egg, situated between the ovary and the abdominal wall, from which they isolated an acid-fast bacillus which grew best at about 25° C. and could also grow at temperatures up to 34° C. On glycerin-serum and glycerin-agar the bacillus formed white rounded knob-shaped colonies or a moist, creamy-white layer, and on glycerin broth a smooth, creamy, easily broken-up pellicle. Cultures on solid media frequently showed a violet discoloration. The strain was pathogenic for carp, frogs, snakes and many kinds of reptiles, but not for guinea-pigs, rabbits or birds (Dubard, 1898).

According to Weber and Taute (1905) the fish tubercle bacillus is identical with the strain obtained by Möller (1898) from a blind-worm and with 36 strains which they themselves obtained from the organs of normal stock frogs, and from the mud, moss and earth on which the latter had lived. Bataillon, Dubard and Terre believed that the acid-fast bacilli were the cause of the tumours in the carp, but Weber and Taute considered that they were more probably saprophytic organisms which had gained access to the tissues of the carp and multiplied abundantly in tumours which were due to some other cause.

Terre (1902) found in another carp from the same hatchery, in addition to an abdominal tumour with acid-fast bacilli, nodules in the liver and testes which also contained acid-fast bacilli. Although Terre examined many carp during the subsequent seven years no further cases were found and he concluded that spontaneous tuberculosis of fish was rare.

Salt-water fish. In salt-water fish living free, spontaneous tuberculosis is also exceedingly rare. Bertarelli and Bocchia (1910) searched for tuberculous lesions among fish exposed for sale in different markets in Italy and failed to find a single example. Three instances only have been put on record. Alexander (1913) found in a cod-fish (*Gadus callarias*) lupus-like lesions associated with acid-fast bacilli. Sutherland (1922) reported a case of extensive generalized tuberculosis in a halibut (*Hippoglossus hippoglossus*). Histologically the lesions were granulomata without typical giant-cells. In neither of the above cases was a culture of the acid-fast organism obtained. The author investigated the roe of a halibut sent to him by Professor S. H. Gaiger, of Liverpool. The roe consisted of translucent grey tissue infiltrated with irregular soft yellow foci about 1 mm. in diameter, which contained acid-fast bacilli. A single colony of acid-fast bacilli was obtained from the roe after treatment with alkali. Cultures of this organism were very different from those of the carp tubercle bacillus and resembled rather closely those of a bovine strain. On egg growth was thin, dry, greyish white, and later showed secondary pearly white colonies. On glycerin-egg growth was better and more granular. On broth it produced an extremely thin, easily broken-up grey film speckled with white foci. The organism grew at 25° C. but not at 37° C. The growth was coherent and difficult to emulsify. The microscopic characters of the bacilli in culture were indistinguishable from those of mammalian tubercle bacilli. In the original material the bacilli varied in length but were mainly long, curved and beaded. The strain was not pathogenic for the guinea-pig or the rabbit. Inoculated subcutaneously into toads the bacilli multiplied in the organs and caused death, but without producing macroscopic lesions. The organism was not pathogenic for gold-fish by feeding (nine months' observation).

In captivity salt-water fish appear to be quite susceptible to infection with acid-fast bacilli. Aronson (1926) examined a large number of salt-water fish which had died in the tanks of the Philadelphia aquarium. He found in the organs numerous disseminated, greyish-white, circumscribed tubercles and nodules, firm and homogeneous in structure, and in one case an intestinal ulcer. These lesions contained masses of acid-fast bacilli, some beaded, some uniformly stained. Identical cultures were obtained from several different species of fish, namely, a serjeant major (*Abudefduf mauritii*), 3 croakers (*Micropogon undulatus*), and 2 sea bass (*Centropristes striatus*). The writer obtained a strain of this organism from the National Collection of Type Cultures, and found it to resemble very closely those isolated from caymans (see page 329). The occurrence of the same type of acid-fast organism in captive reptiles as in captive fish suggests that this type of bacillus is ordinarily a saprophyte of mud and slime, which is capable of parasitizing cold-blooded animals living under the unnatural conditions obtaining in reptile houses and aquariums. Aronson has suggested for his strain the name *Mycobacterium marinum*, but this name seems hardly appropriate since the fish became infected in captivity.

Frogs.

Rupprecht (1904) described in the liver of a frog yellowish-white nodules up to 2 and 4 mm. in diameter, which were said to resemble secondary carcinomatous nodules in man. They contained masses of acid-fast bacilli mainly intracellular. Küster (1905) found lesions in the livers of three frogs and obtained cultures which on serum resembled the avian tubercle bacillus and on agar formed a drier growth recalling the human bacillus. The strains were highly pathogenic for frogs. Lichtenstein (1920) reported a similar case. N. S. Lucas discovered in the liver of a paradoxical frog (*Pseudis paradoxa*), which died in the gardens of the Zoological Society of London, numerous soft white foci composed almost wholly of a small acid-fast bacillus. The author (1928) obtained a culture which grew at 25° C. but not at higher temperatures; it produced on glycerin-egg, agar and broth, moist white or slightly creamy layers resembling cultures of the avian tubercle bacillus. The culture was pathogenic for frogs and toads.

Turtles.

Friedmann (1903) described two cases of spontaneous pulmonary tuberculosis in turtles (*Chelonia corticata*). The disease was confined to the lungs, which in one case were riddled with cavities. The lesions contained enormous numbers of acid-fast bacilli. In sections of some parts of the lung Friedmann found tubercles which presented the typical histological picture of mammalian tuberculosis. The bacilli were pathogenic for the frog, turtle, lizard and common snake. From a third case of tuberculosis in a turtle, Friedmann obtained a strain which was a little different from the first and resembled the fish tubercle bacillus.

Wilson (1925) obtained a culture from a tuberculous terrapin which died in the Zoological Society's Gardens, London. The culture was sent to the author, who separated from it two acid-fast strains, one of which produced moist orange-yellow, easily emulsified cultures, while the other formed dry white coherent growths, which could not be spread or emulsified ; the yellow pigment was soluble in alcohol and ether but not in xylol. The culture grew at a wide range of temperature up to 37·5° C.

Snakes.

Sibley (1889) reported a case of general tuberculosis in a ringed snake (*Tropidonotus natrix murorum*). There were caseous tumours in the subcutaneous tissues, in the body cavities along the vertebral column and in the organs ; the lesions, composed of small round cells, necrosed in the centres of the larger tumours, contained numerous acid-fast bacilli. Another case was reported by Sibley (1891) in a viper ; he found in the subcutaneous tissues of the neck a large encapsuled tumour consisting of dry caseous masses. Gibbes and Shurly (1890) found tuberculomata in the livers of a boa and a python ; the lesions were very rich in acid-fast bacilli. Shattock (1892) and Hansemann (1903) each described one case of tuberculosis in a python. In none of the above cases were the bacilli investigated bacteriologically. Aronson (1929) described four cases of spontaneous tuberculosis in garter-snakes (*Thamnophis sirtalis*) which occurred in the Philadelphia Zoological Garden. The livers were studded with firm grey-white tubercles, 1 to 2 mm. in diameter ; the right lung in three of the snakes showed numerous small areas of consolidation ; in one snake there was an abscess near the anterior pole of the liver. The lesions contained numerous acid-fast bacilli. Cultures were obtained from three of the snakes ; the strains were identical culturally, producing on egg pinkish and salmon-coloured colonies, and in antigenic properties, but differed in these respects from certain other pathogenic acid-fast bacilli from cold-blooded animals. The bacilli were acid- but not alcohol-fast. Aronson considers the organism to be a new species for which he proposes the name *Mycobacterium thamnopheos*. H. H. Scott found tuberculous lesions in a snake (*Tarbophis fallax*) which died in the Zoological Society's Gardens, London. The lesions were small caseous masses situated in the mesentery, particularly abutting on the spine, and also in the lungs ; they contained numerous well-stained acid-fast bacilli. Griffith (1928) obtained a culture and identified it with the fish tubercle bacillus of Bataillon, Dubard and Terre.

Alligators.

N. S. Lucas and H. H. Scott discovered tuberculosis in four rough-eyed caymans (*Caiman sclerops*), which died in the gardens of the Zoological Society, London. The appearance and distribution of the lesions were very similar in the four animals. The livers and spleens showed numerous discrete soft white caseous tubercles and there were similar but less numerous tubercles in the lungs. The lesions contained numerous acid-fast bacilli, mainly long, curved and vacuolated. Griffith (1928) obtained a culture from each cayman and found the strains to be identical. They produced on solid glycerin media and on glycerin broth soft thick canary yellow, creamy or buff layers which on glycerin-egg were often slimy and tenacious. The yellow pigment was soluble in alcohol and ether but not in xylol. The cultures were pathogenic for frogs and toads, but not for the guinea-pig.

Summary of Characters of Acid-fast Bacilli from Cold-blooded Animals.

Morphological characters. In the tissues and on culture, the acid-fast bacilli of cold-blooded animals exhibit the same range of variation in size, shape and granularity as the tubercle bacilli of higher vertebrates. Some strains show bulbous and filamentous forms in old cultures; branched forms have also been described.

Staining reactions. The bacilli readily stain by the Ziehl-Neelsen method in the cold and are strongly acid-fast. They also stain by Gram's method.

Cultures. The bacilli are aerobic and grow best at 25° C.; some strains can grow at higher temperatures up to 37° C. They do not liquefy gelatin or coagulate milk; the latter, however, may be thickened. The strains grow on the surface of broth, producing pellicles of varying thickness, which are easily broken and tend to fall to the bottom of the fluid, where they form an abundant flocculent deposit; the broth remains clear. Some cultures have a pleasant aroma not unlike tuberculin. Growth is favoured by the addition of glycerin and glucose to media. On solid media some strains are dry, coherent and difficult to emulsify; others produce moist and tenacious, or soft and easily emulsified growths. Some strains produce pigment; others have white or translucent growths.

Vitality of cultures. The bacilli remain alive in cultures for very long periods. A primary culture on glycerin-egg of the frog strain produced good subcultures when it was four years old, and other strains have remained viable for a year or more.

Pathogenicity. The acid-fast bacilli of cold-blooded animals are not pathogenic for mammals or birds. When inoculated in large dose into the dorsal lymph sac of the frog or the toad they multiply abundantly in the tissues, especially the liver and the spleen, and cause death without provoking obvious lesions. In toads which die several months after inoculation the liver often shows a grey dust-like speckling. All the strains which have been obtained from cold-blooded animals appear to have this power of multiplication in the bodies of frogs and toads after parenteral inoculation. The fish tubercle bacillus has shown similar pathogenicity for the carp and for many other cold-blooded species of animals. In order to compare the infectivity of strains from different sources the natural mode of infection in these animals, that is by feeding, should be employed. The frog strain administered in this way multiplied in the tissues of frogs, but did not produce lesions within 223 days. The frog, snake, cayman and halibut strains, given as food or added to the water as an emulsion, were not pathogenic for gold-fish under observation for 9 to 12 months. Some of the gold-fish died prematurely, and acid-fast bacilli identical with those given as food were demonstrated by culture to be present in the tissues in small numbers.

Tuberculin. Discordant results have been reported in regard to the production of tuberculin, but Borrel (1904) and Weber and Taute (1905) have shown that the products of the growth of the bacilli (carp and frog strains) as well as the bodies of the bacilli are not toxic for the tuberculous guinea-pig. Borrel found that a dose of 600 mgm. of the fish tubercle bacillus was harmless for the tuberculous guinea-pig, while 20 mgm. of human bacilli killed a tuberculous guinea-pig in the same stage of tuberculization.

Serological reactions. The acid-fast bacilli of cold-blooded animals do not form a homogeneous group and can be divided into serological types. At least four types (see Table I) have been differentiated by agglutination and absorption tests.

TABLE I.

Type I.	Type II.	Type III.	Type IV.
Cayman strains. *Mycobacterium marinum* (Aronson).	Frog strain.	Snake strain. Carp strain. Turtle strain. (Friedmann).	Halibut strain.

Wilson (1925) classed together the fish (carp) and turtle strains and found, as did the author, that neither the terrapin (yellow) nor the frog strain removed the agglutinin from the fish tubercle serum. The terrapin strain was agglutinated by the cayman serum but did not remove the homologous agglutinin. It would be of interest to compare the serological reactions of pathogenic acid-fast bacilli with those of saprophytic acid-fast strains obtained from normal organs of frogs, &c., and from mud and slime.

Passage experiments. Many of the earlier investigators believed that they had been successful in transforming tubercle bacilli of mammals or birds by passage through the bodies of various cold-blooded animals into bacilli having the characters of the fish tubercle bacillus. These results have not been confirmed by later investigators, and it seems certain, in the light of the findings of Weber and Taute and Petrow, that the earlier observers cultivated acid-fast bacilli of the fish tubercle group, which by chance had found their way into the tissues of the experimental animals, the inoculated tubercle bacilli having died out.

Tubercle bacilli of the higher vertebrates are not pathogenic for cold-blooded animals. They can, it is true, when inoculated into the dorsal lymph sacs of frogs and toads, multiply for a time at the seat of inoculation and in the organs of the body, especially the liver and the spleen, but they do not produce true tuberculosis and gradually die out. At the seat of inoculation there may be found a caseating lesion or a granuloma.

Bertarelli and Bocchia (1909–10) found that bovine, human and avian bacilli could multiply in the body of the carp or lizard (*Varanus varnis*) but remained unchanged in virulence when recovered 8 months later. Sion (1900) reported similar results in frogs after 6 months' residence.

The author passed a strain of bovine tubercle bacilli through the bodies of a succession of toads, the total period of residence in the toad body being nearly 8 years. One of the toads was allowed to live 3 years, and when killed showed numerous tubercle bacilli at the seat of inoculation and in the liver and spleen. The bacilli were, however, nearly all dead, and only a few from the liver were able to produce colonies in culture. After this prolonged sojourn the bacilli at the end of the experiment were unchanged in cultural characteristics and in virulence.

A strain of avian type was unchanged in cultural characters after residing 2½ years in the body of a toad.

The acid-fast bacilli of cold-blooded animals are not pathogenic for the animals of constant temperature, and cannot be converted into ordinary tubercle bacilli. The apparent conversion of the carp strain in guinea-pigs, which was reported by Bataillon, Dubard and Terre, must be attributed to the use of a mixed virus, the mammalian tubercle bacilli being derived from the water, contaminated with tuberculous sputum, in which the carp lived.

REFERENCES.

Alexander, M., 1913, *Trans. Lpool Biol. Soc.*, **27**, 219.

Aronson, J. D., 1926, *J. Infect. Dis.*, **39**, 315; 1929, *ibid.*, **44**, 215.

Bataillon, Dubard & Terre, 1897, *C.R. Soc. Biol.*, Paris, 10 s., **4**, 446.

Bertarelli, E. & Bocchia, J., 1910, *Zbl. Bakt.*, Abt. I, Orig., **54**, 385.

Borrel, A., 1904, *Bull. Inst. Pasteur*, **2**, 409, 457, 505.

Dubard, 1898, *Congrès pour l'étude de la tuberculose*, Paris, 711.

Friedmann, F. F., 1903, *Zbl. Bakt.*, Abt. I, Orig., **34**, 647; 1904, *Deuts. med. Wschr.*, **30**, 166.

Gibbes, H. & Shurly, F. L., 1890, *Amer. J. Med. Sci.*, **100**, 151.

Griffith, A. S., 1928, *J. Hyg.*, Camb., **28**, 198.

v. Hansemann, 1903, *Zbl. Bakt.*, Abt. I, Orig., **34**, 212.

Küster, E., 1905, *Münch. med. Wschr.*, **52**, 57; 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, **5**, 746; 1928, *ibid.*, hrsg. Kolle, Kraus u. Uhlenhuth, **5**, 1037.

Lichtenstein, S., 1920, *Zbl. Bakt.*, Abt. I, Orig., **85**, 249.

Möller, A., 1898, *Ther. Mhft.*, **12**, 607.

Petrow, N. P., 1907, *Zbl. Bakt.*, Abt. I, Orig., **43**, 349.

Rupprecht, 1904, *Inaugural Dissertation*, Freiburg, 1.

Shattock, S. G., 1902, *Trans. Path. Soc. Lond.*, **53**, 430.

Sibley, W. K., 1889, *Virchows Arch.*, **116**, 104; 1891, *Brit. Med. J.*, **1**, 11; 1892, *Trans. Path. Soc. Lond.*, **43**, 189.

Sion, V., 1900, *Zbl. Bakt.*, Abt. I, **27**, 710.

Sutherland, P. L., 1922, *J. Path. Bact.*, **25**, 31.

Terre, L., 1902, cited from 1903, *Zbl. Bakt.*, Abt. I, Ref., **33**, 200.

Weber, A. & Taute, M., 1905, *TuberkArb.*, Berl., p. 110.

Wilson, G. S., 1925, *J. Path. Bact.*, **28**, 69.

CHAPTER VI. JOHNE'S DISEASE.

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Synonyms. Chronic paratuberculous enteritis of cattle, l'entérite paratuberculeuse des bovidés, chronische paratuberkulöse Darmentzündung beim Rinde.

Definition. Johne's disease is a chronic infectious disease of cattle, characterized by a diffuse thickening and corrugation of the intestine and caused by a specific organism, the Johne bacillus (*Mycobacterium paratuberculosis* Bergey).

History.

Although Hutrel d'Arboval (1826), Farrow and Cartwright, Hansen and Nielsen, all recorded conditions which can now be recognized as Johne's disease, it was Johne and Frothingham (1895), who discovered in a typically altered intestine the acid-fast bacillus since found to be characteristic of the disease, and gave a detailed description of the macroscopic and microscopic characters of the lesions and negative results of guinea-pig inoculation. They regarded the bacilli as tubercle bacilli, possibly of avian origin. In 1904, Markus described several similar cases and introduced the method of diagnosis by the microscopic examination of rectal mucous membrane.

Numerous reports followed, but it was B. Bang who, in 1906, first recognized that the disease was a distinct one, adduced definite evidence for his conclusion and showed the possibility of reproducing the disease by administering infected mucous membrane to calves. In 1907, M'Fadyean published a full account of 9 cases in England, suggesting the name 'Johne's disease' and giving details of the lesions, histology, and negative results of cultivation and inoculation experiments on guinea-pigs and rabbits. Bang's original transmission experiments were confirmed by Miessner and Trapp (1910) who also succeeded in infecting calves by intravenous inoculation. The next real advance in our knowledge of the disease was made by Twort and Ingram (1910–13), who artificially cultivated the bacillus and succeeded in reproducing the disease with cultures. They also made the first attempts to prepare a specific diagnostic agent analogous to tuberculin. From 1912–16, five publications appeared by M'Fadyean, Sheather and Edwards, dealing very fully with the disease, including methods of infection, cultivation and diagnosis.

Distribution.

The disease has been described in nearly every country of Europe, in North America, South Africa and India, Australia and New Zealand. It is usually sporadic but on certain farms and in various countries, particularly Switzerland, Denmark and England, it has at times become enzootic.

Morphology and Staining of the Johne Bacillus.

The causal bacillus is a non-motile, non-sporing, acid- and alcohol-fast bacillus, nearly 1 to 2 × ·5μ ; it stains evenly, but the longer forms occasionally found tend to show alternate light and dark portions. *Staining* is best carried out by Ziehl-Neelsen's method or one of its modifications, but may also be effected by a modified Gram's method provided staining is sufficiently prolonged. In affected tissues there is a marked tendency to clump formation.

Cultivation.

The bacillus can be isolated from lesions only on media to which have been added certain other acid-fast bacteria, their products or extracts. The earlier subcultures must be carried out on media with a similar addition, but later it is sometimes possible to train the bacillus to grow without the factor already elaborated by other acid-fast bacteria. Growth on any medium is slow, particularly in the earlier generations.

The medium on which Twort originally cultivated the bacillus was composed of egg, glycerin (5 per cent.), and killed ground human tubercle bacilli (1 per cent.), the mixture being coagulated at 85° C. Subsequently other members of the acid-fast group have been used to supply the already elaborated factor required for growth : *B. phlei* is particularly to be preferred, as it grows luxuriantly and its extracts evoke no allergic response in animals affected with Johne's disease (O. Bang, 1911 ; Vallée and Rinjard, 1926), an important fact when johnin is being prepared. M'Fadyean, Sheather and Edwards (1912) and Holth (1912) showed independently that the bacillary bodies may be replaced by a glycerin-saline extract or by the products produced during the growth, and further that, with such addition, egg, agar, serum and broth are suitable media. Dunkin (1928) obtained good results with a solid medium containing egg, liver-extract, glycerin and killed *B. phlei* to which was finally added gentian violet to 1 in 10,000, and a satisfactory johnin from the abundant surface growths obtainable on a liquid medium made of tryptic digest horse-muscle broth, potato-extract, liver-extract, glycerin and killed *B. phlei*. Satisfactory growth may be obtained on a synthetic asparagine medium containing dead *B. phlei* or its extracts.

The Johne bacillus is aerobic, the optimum temperature for multiplication is 39 to 40° C. (limits 30 to 43° C.) ; growth is reported at pH 6·8 to nearly 8·0.

PRIMARY ISOLATION.

Selected material is well triturated with sand, treated with a 20 per cent. concentration of antiformin for 20 minutes, immediately well diluted and centrifuged ; the deposit is washed and sown on suitable media, preferably with an egg basis. The presence of gentian violet makes the early colonies more visible, and also, according to Petroff, who originated the use of this dye in the isolation of tubercle bacilli, prevents the growth of many of the common contaminants, without hindering the development of acid-fast organisms. Cultures from glands may be obtained without the use of antiformin.

CHARACTERS OF CULTURES.

On egg media growth usually appears after 3 to 4 weeks' incubation ; in primary cultures it may be continuous, but generally colonies are discrete, colourless and slightly opaque, the opacity increasing gradually until they may be distinctly whitish. Very often in primary cultures and often in subsequent generations on this and other media, secondary colonies appear, which are larger and more raised, becoming definitely opaque and white or slightly yellow. Subcultures from the latter and from the ordinary smaller colony are similar, usually continuous and soon become slightly opaque and whitish.

On transparent media such as glycerin agar, separated colonies are slightly greyish or brownish, and become more so with increasing age : they are convex, and when magnified are seen to have a central yellowish or brownish opaque portion surrounded by a thin shaggy edge.

Broth. Surface cultures are difficult to start ; usually some agent is necessary to enable the seed material to float and yet be in contact with the medium. Should it sink growth occurs slowly in the form of hard granules. A surface inoculum spreads slowly to form a thin membranous uniform greyish film, which later develops small thicker spots or streaks of whitish appearance. Subsequent transfers are generally better and may become abundant, thick and wrinkled, then resembling a surface growth of tubercle bacilli.

Pathogenic Action.

NATURAL AND ARTIFICIAL INFECTION.

Natural infection. Bovines are the animals usually affected and the species in which the disease is preserved. Symptoms occur most commonly in cattle aged 3 to 6 years; young animals are probably the most susceptible, but the period of incubation is very prolonged, probably 2 to 3 years or longer. Natural infection of sheep and goats has been recorded by M'Fadyean and Sheather (1916) and of a deer by M'Fadyean (1907).

Infection is derived from a preceding case by contamination of food, water or bedding, with fæces containing bacilli : pastures may become badly contaminated and infection occurs through that medium, as the bacillus is somewhat resistant to the action of light and desiccation. Its resistance to antiformin, carbolic acid and other disinfecting agents is at least equal to that of the tubercle bacillus.

Artificial infection. The typical disease has been reproduced in a cow, aged 8 years, and in calves, sheep and goats, by intravenous inoculation or feeding with material from lesions or cultures. Experiments on two donkeys and a pony had a negative result.

The experiments on calves of M'Fadyean and Sheather (1916), Meyer (1913), and of the earlier workers tend to show that it is the very young animal which is most susceptible ; at 6 months calves are already becoming more resistant and in animals of 12 months or over the only instance of artificial transmission is that recorded by Twort. Experiments with large intravenous doses on calves aged 3 months or less have nearly all been successful, whereas at least half the animals between the ages of 6 and 12 months, given large amounts of infective material or doses ranging up to 1,000 mgm. of culture and killed 1 to 2 years later, have shown no evidence of disease ; in the remainder the infection has still been in the incubative stage, macroscopic lesions being usually slight or absent and bacilli often scanty. Infection by ingestion is rather more difficult ; nevertheless, that is undoubtedly the natural method of transmission and probably occurs early in life : the number of bacilli ingested would be relatively small and the period of incubation distinctly longer.

Laboratory animals. C. C. Twort and Craig (1913) compared intravenously in rabbits the toxicity of the Johne bacillus and numerous other acid-fast bacilli in doses varying from 30 to 200 mgm. of pure culture. It was found that the Johne bacillus was amongst the least toxic, a single dose causing neither rise of temperature, loss of appetite or weight, nor lesions other than a transient congestion of kidneys and lungs. A second dose, particularly if given 15 to 30 days later, or repeated small doses, produced a more serious reaction ; many animals died with acute lung and kidney lesions, or when killed at various intervals showed tubular nephritis and lung foci resembling the early stage of a miliary tubercle. Intraperitoneal or subcutaneous inoculation produced only local caseous lesions in which the bacilli were apparently dead, but persisted for a long period.

In 1914, C. C. Twort described further experiments on 81 animals, rabbits, mice, guinea-pigs and rats by the intravenous, intraperitoneal, oral or subcutaneous route. The strain chiefly used was one acclimatized to ordinary media. When killed 2 to 6 months later about half of the rabbits inoculated intravenously and a fifth of the rats and mice inoculated intraperitoneally showed evidence of intestinal infection. The lesions were small in extent, but bacilli were present in small or large number and could also be demonstrated in liver and spleen. Their persistence in the glands or peritoneal cavity of animals showing no intestinal infection was marked. Some evidence was obtained *in vitro* of their low toxicity for leucocytes. Boquet (1925) obtained similar results in rats.

Finally, it may be said that the changes caused in small animals by large doses of bacilli bear small likeness to the natural disease, but are valuable in shedding light on the toxicity of the Johne bacillus and its marked resistance to destruction in the animal body.

MORBID ANATOMY.

The association of diarrhœa, continuous or intermittent, and emaciation with a normal or rapacious appetite is the most conspicuous symptom and probably due to the action of the bacilli and lesions in interfering with the normal absorption of food.

Lesions are always diffuse, never nodular or caseated, and are confined to the intestine and its glands. They are most frequent in the neighbourhood of the ileo-cæcal valve, and may be evenly distributed, but more often are of a somewhat patchy character.

The small intestine, particularly the ileum, tends to show the most frequent and marked alterations, usually in the form of a more or less pronounced thickening and corrugation, longitudinal or transverse, and not removed by moderate stretching. In the large intestine and cæcum these changes are usually less distinctive but congestion is more frequent. Apart from this, the surface of all affected parts is generally of a greyish white colour and covered with a thick layer of mucoid material.

The ileo-cæcal valve is often markedly enlarged, of a cherry red colour, wholly or in part, and may be the only place in which lesions or bacilli are demonstrable. The rectum may show definite changes, although the latter part or even the whole of the large intestine is unaffected. The mesenteric and colic glands are frequently enlarged and œdematous. Distinct congestion, together with a small increase of mucoid material, may be the only alteration, or occasionally, no macroscopic lesions may be found.

Microscopic examination of smear preparations will demonstrate the specific acid-fast bacillus, but in very variable numbers: the average richness of 20 to 30 smears taken from various parts of the intestine bears no relation to the clinical history, nor does the number demonstrable in a single preparation depend on the lesions present at the place from which it was taken.

MORBID HISTOLOGY.

The mucous membrane is primarily affected and may be the only part altered, but changes are generally also present in the submucous layer, producing the characteristic macroscopic thickening and wrinkling. The first distinct alteration is a gradual infiltration of the pre-existing tissues with cells of the epithelioid or plasma type; as a result the villi are thickened, often stunted; they may be in part denuded of their epithelium. The interstitial tissue is increased, frequently to an advanced degree, and as the disease progresses the normal tissue elements appear to become atrophied. It is this disappearance of glands and interference with the normal intestinal functions which probably causes the progressive emaciation. At a later stage the boundaries of the newly formed cells disappear, and an incomplete reticulum is seen, in which the remains of cell boundaries and degenerate nuclei can be made out. The bacilli may be quite numerous and well stained at this stage; at no period are they noticeably intracellular and when numerous are generally disposed in clusters, which may be so extensive that a stained section appears distinctly red. Giant cells are occasionally present in sections of intestine and more often in those of gland.

The most noticeable fact in the histology of this disease, which so far is comparable to that of a tuberculoma, is that the lesions, although gradually extending, do not progress to caseation. The probable reason is that either the bacilli do not excrete any potent cell-poison or they are resistant to the action of the body-cells and fluids, in consequence of which no large amount of endotoxin is liberated at any one time by the death and disruption of bacilli. Caseous lesions, apparently due to Johne bacilli and not to tubercle bacilli, do, however, occur as a result of the inoculation of certain resistant animals, e.g. the guinea-pig and rabbit; in such lesions, all or the majority of the bacilli are dead. M'Fadyean and Sheather (1916) have reported caseous lesions containing living Johne bacilli in the mesenteric glands of goats, whose intestines also showed bacilli but no lesions.

Diagnosis.

MICROSCOPIC EXAMINATION.

Pieces of rectal mucous membrane may be obtained, well washed, stained and examined for the typical clumps of small acid-fast bacilli; a positive result is strongly indicative of infection, whilst a negative one, often obtained even in advanced cases, is of no significance. The finding of typical clumps of bacilli in preparations of fæces has also a positive significance. The examination of smears of untreated material is of doubtful value; the numbers of bacilli voided are variable and acid-fast saprophytes may be present. The latter can be dissolved, according to Meyer (1926) and Miessner and Erichsen, by the use of 50 per cent. antiformin, the Johne bacilli being at the same time concentrated.

SEROLOGICAL REACTIONS.

The agglutination and complement-fixation methods are of no practical value. Cultures of the Johne bacillus (Twort and Ingram, 1913), avian tuberculin (O. Bang and Anderson, 1913), an extract of affected mucous membrane (Miessner and Kohlstock, 1912) and a methyl alcohol extract (Boquet) have each been used as antigen. Sera from affected animals are of low titre and react almost to the same degree with antigens from the Johne bacillus, *B. tuberculosis*, and other acid-fast bacteria.

ALLERGIC REACTIONS.

The agents used to demonstrate a specific or group specific hypersensibility are avian tuberculin, and johnin, both substances analogous to ordinary tuberculin.

Avian Tuberculin.

In 1909 and 1910, O. Bang reported that bovine animals affected with Johne's disease, which gave no reaction to 20 c.cm. of ordinary tuberculin, responded to the subcutaneous injection of 2 c.cm. of avian tuberculin prepared in a similar manner. This was based on tests of 1,700 animals either showing clinical symptoms or forming part of infected herds; in the latter, 6 to 35 per cent. of adults and about 2 per cent. of animals under two years, reacted. The results of other workers have been similar and have in some cases shown that by the removal of all reactors to this agent the appearance of clinical cases could be prevented. Unfortunately, however, tuberculous subjects may react and that fact has induced subsequent investigators to use an agent prepared from the causal bacillus. Further, Schalk (1926) has shown that cattle may, by cohabitation with tuberculous fowls, become sensitized to avian tuberculin. Plum (1925) has demonstrated the invasion of cattle under natural conditions by the avian tubercle bacillus.

Twort and Ingram (1913) were the first to prepare a diagnostic agent, johnin, prepared from the causal bacillus and in 1916 the results of a large number of detailed experiments were published by M'Fadyean, Sheather and Edwards. The latter authors came to the conclusion that animals affected with tuberculosis might also react, but this opinion has not been universally accepted, and other workers have collected much evidence to the contrary. It is to be noted, however, that the later workers have not, as have the authors cited, performed tests on calves artificially infected with tubercle or Johne bacilli.

Johnin.

Preparation. Surface cultures on broth are the most convenient because profuse growth can be obtained and the first essential to the production of a potent johnin thus ensured. Extraction can be carried out by heat or in the cold. Twort and Ingram (1913) using surface broth cultures and Beach and Hastings (1922) using growth in broth, extracted by heating at 60 to 65° C. for 1 hour. M'Fadyean, Sheather and Edwards (1916) used surface broth cultures, washed twice to free them of the extract of human tubercle bacilli added to assist growth, and doubly extracted, once by steam and once by autoclaving at 115° C. Dunkin (1928), used surface growths on broth and dead *B. phlei*, extracted and killed during concentration to 1/10. Vallée and Rinjard (1926), however, state that in their hands an extract of the bacilli and medium of culture (serum glycerin agar with *B. phlei*), made in distilled water in the cold, has given better results than any other and constitutes a good reagent, producing marked reactions in vaccinated animals and smaller but distinct ones in clinical cases. Wright (1928) and Turner (1928) prepared johnin from a surface culture on Long's synthetic medium, by 'plasmolysing' it with ether and extracting with distilled water, each process being carried out, without heat, three times and for three days each time.

The routes employed have been subcutaneous, intravenous, intradermal and ophthalmic. Reactions tend to be of a smaller degree than those elicited by tuberculin in tuberculous subjects, and, in extreme cases, there is a similar relative inaptitude to react, necessitating larger doses and earlier recording of results.

For the subcutaneous test 2 to 5 c.cm. are injected and results read from the subsequent temperature chart, marked every 2 to 3 hours from the sixth to the eighteenth hour or later.

Intravenously 3 to 10 c.cm. are given, and temperatures recorded every 1 to 2 hours up to 12 hours, the maximum temperature being generally reached between the fifth and eighth hour.

By either method a rise of 2° F. above the pre-inoculation temperature is the least that can be interpreted as of positive significance, though reactions are frequently greater and range up to 5° F. In about 25 per cent. of animals injected intravenously and in a smaller number subcutaneously injected, some constitutional reaction occurs, most commonly in the form of diarrhœa, but occasionally as staring coat, muscular trembling and prostration. Such reactions, which commence during the first four hours after intravenous injection and a little later after subcutaneous injection, are of positive significance; diarrhœa may last 24 hours.

For the intradermal test about 0·2 c.cm. of johnin concentrated to a tenth of its volume or a larger volume if dilute, is injected into the anal fold or preferably into a small shaved area. Caliper measurements are taken 24 and 48 hours afterwards; in the case of the anal fold comparison may be made with the fold of the opposite side. Account must also be taken of the character of the swelling, which in a positive reactor is diffuse, often feather-edged, hot and tender. Particularly will diagnosis be thus aided if the reaction is only of a moderate degree, i.e. 2 to 3 times the original thickness. Changes occurring before the twenty-fourth hour and of a transient nature should be disregarded. According to Dunkin (1928) it is a definite advantage to inject a further 0·2 c.cm. at the same place 48 hours later, if the reaction to the first is not already diagnostic; readings are then made at 72 and possibly at 96 hours.

The ophthalmic method carried out with a single instillation has not proved of value: a double ophthalmic method has, however, been used by Vallée and Rinjard (1926).

The value of johnin depends on the extent to which it is specific and able to detect animals in which the disease is still in the incubative stage, but whose fæces may contain the specific bacillus. Unfortunately, there is little satisfactory evidence to show the relation between the presence of bacilli in the fæces, the development of specific hypersensibility and the time since infection. It has, however, been shown by Twort and Ingram (1913) and by M'Fadyean, Sheather and Edwards (1916^2) that artificially infected animals usually react early in the incubation period. Moreover, there is general agreement that most clinical cases give a definite response ; the fact that advanced subjects may fail to react is not a serious difficulty, because such cases can be recognized otherwise and would be removed from the herd. Normal animals give no reaction to johnin ; this is well shown in herds in which the history shows that Johne's disease has been absent or very infrequent. So definitely do Vallée and Rinjard hold this view concerning their own form of johnin, that they regard any reaction, not of an early or transient character, as evidence of infection.

M'Fadyean, Sheather and Edwards (1916^2) reported subcutaneous tests of 13 advanced clinical cases and obtained reactions in 8.

Dunkin (1928) using the double intradermal method, carried out 150 tests on 36 clinically affected animals and, with the exception of a few animals *in extremis*, all responded. This observer also reports the results of tests of 58 animals suspect of Johne's disease, in various parts of England. In 28 (49 per cent.), the result of the test was in agreement with the post-mortem findings, or with the clinical data at the time of test or subsequently ; in 7 (12 per cent.) it was not in agreement, whilst in the remaining 22 (39 per cent.) no conclusion could be formed, as the animals were still under suspicion or no report had been received.

Wright (1928), using 5 c.cm. intravenously, reports tests on 343 animals in known infected herds : 91 (26·5 per cent.) were positive, 51 (14·8 per cent.) ' suspicious ' and 198 (56·2 per cent.) negative, whilst in 8 (2·3 per cent.) the test was regarded as unsatisfactory owing to high pre-inoculation temperature. Although the results show that a large proportion of animals give no reaction, there are too few autopsies to make possible a correct evaluation of a positive response.

Ernest (1927) using the intravenous method, on a herd of 207 Jersey and Ayrshire cows, in which the loss from Johne's disease had been serious, obtained positive reactions in 40 per cent. of the total animals : 6 reactors, including 2 calves, were killed and all showed evidence of infection. No reference is made to tuberculosis.

Beach and Hastings (1922) used 10 c.cm. intravenously, and, after some preliminary work on the specificity and constancy of the reaction, tried to eradicate the disease in certain herds. One herd, averaging 50 cows, in which the disease had caused serious annual loss for 14 years previously, was tested 9 times in 4½ years with the following reactors: 5, 4, 6, 4, 4, 4, 6, 0, 0. In 29 out of 30 cases investigated *post mortem* it is stated that clear evidence of disease was found : 20 of these showed either slight or marked lesions of the intestine or ileo-cæcal valve, and, in those that showed no lesions, acid-fast bacteria were demonstrated in material obtained from small pieces of mucous membrane by drying, powdering and treating with antiformin. No clinical cases developed after the first test.

Tuberculous herds. Turner (1928), using 5 c.cm., intravenously tested 20 animals in a herd of 45 tuberculous cows, believed to be free from Johne's disease. Two animals, afterwards found to be suffering from advanced tuberculosis, reacted : on the other hand, 12 non-reactors were submitted to post-mortem examination and all showed tuberculous lesions, in two cases generalized. In the two reactors, no lesions of Johne's disease were reported and no attempt was made to demonstrate the specific acid-fast bacillus by the microscope or culture.

Dunkin (1928) tested 49 animals, in a herd badly infected with Johne's disease, simultaneously with tuberculin and johnin, in each case by the double-intradermal method ; of these animals 8 reacted to johnin alone, 8 to tuberculin alone, 13 to both tests and 20 to neither. Eleven animals, selected from the four groups, were submitted to post-mortem examination, including cultural investigation and the findings were in agreement with the tests.

Immunization.

According to the preliminary experiments of Vallée and Rinjard (1926), it seems possible that calves can be protected by the subcutaneous injection of Johne bacilli, 5 to 15 mgm., incorporated in an unabsorbable excipient composed of oil of vaseline and finely powdered limestone. In previously healthy animals, which should preferably be calves or at least animals negative to johnin, a permanent focus is formed, which, at first œdematous, gradually becomes fibrotic and remains for a period of 1 to 2 years ; during that period animals react definitely to johnin but not to tuberculin, human or bovine. At autopsy the lesion is found to be caseo-fibrotic and from 150 to 200 gm. in weight ; massive quantities of the caseated material appear to have no injurious effect on guinea-pigs or bovines.

Having shown the inocuity of such injection on 16 calves previously tested with johnin with negative results, Vallée and Rinjard treated 277 animals in the field, leaving one-third of that number as controls. After 1 year it was clear that the vaccine had had no injurious effect ; moreover, the results were in favour of the view that vaccinated animals had been protected, e.g. on one farm three controls had become affected and no vaccinated animals and on another some of the controls had become affected, but only one vaccinated animal had to be killed because of Johne's disease, and in that animal no trace of an inoculation lesion could be found.

In animals already affected, the vaccine is quickly expelled without producing any permanent lesion. Full details are not given in this preliminary report of Vallée and Rinjard, and further work is necessary before the method can be satisfactorily evaluated.

Chemotherapy.

No specific or other therapy of much value has been found. M'Fadyean, Sheather and Edwards (1915) tried iron and sulphuric acid *per os* and Sheather (1928) formalin intravenously ; the most that can be said is that these agents appeared to control the diarrhœa for a short period and, in certain cases, to be associated with definite improvement in condition, but a relapse, which did not respond to further treatment, almost invariably followed.

Control.

There is some reason to believe that the incidence of Johne's disease is increasing and that it is becoming a serious menace in this and other countries. The increase of reported clinical cases is probably not now due, as it was in the past, to more accurate diagnosis. It has been shown that in herds in which infection is not of recent origin from 10 to 40 per cent. of the animals will react to the johnin test, whilst in most herds an occasional clinical case occurs. Recent infection of a herd can generally be traced to the introduction of an animal from another herd in which the disease is known to be existent. At the present time, two methods of control are being tried, one based on immunization and the other on the detection of incipient disease by johnin.

Based on johnin tests the following measures may be adopted : (1) segregation or slaughter of all clinical cases, and of suspect cases in which allergic or other means of diagnosis have been positive ; (2) testing of all ' contacts ', followed by segregation if found positive ; such animals should, if practicable, be fattened ; (3) prevention of infection from outside sources by testing all animals before admitting them to the clean herd ; (4) retests after a period of not more than 12 months and preferably after 6 months with removal of positive animals ; (5) prevention of infection of calves, by segregating them as soon as possible after birth, or, failing that, by keeping them in contact only with animals believed to be clean ; (6) thorough periodic disinfection of all stalls and boxes, especially if they are to be used for calves.

Such measures are not easily applied, particularly on small farms. A method based on *immunization*, although requiring somewhat similar measures, has the definite advantage that calves which have developed a satisfactory local lesion as the result of vaccination are protected, and can, therefore, be allowed to mix with the general herd. Further tests of this method must be awaited before conclusions can be formed regarding the extent and duration of the immunity produced by the vaccination. Although the work already done on control by methods based on johnin tests is encouraging, further work, adequately controlled by post-mortem investigation, is required, particularly in tuberculous herds and with artificially infected animals, before the method can be regarded as of proved value.

REFERENCES.

Bang, B., 1906, *Berl. tierärztl. Wschr.*, p. 759.

Bang, O., 1909, *Zbl. Bakt.*, Abt. I, Orig., **51**, 450 ; 1910, *Maanedskrift for Dyrlaeger*, **21**, 561, cited from *Deuts. tierärztl. Wschr.*, **19**, 54.

Bang, O. & Andersen, C. W., 1913, *Zbl. Bakt.*, Abt. I, Orig., **69**, 517.

Beach, B. & Hastings, 1922, *J. Infect. Dis.*, **30**, 68.

Boquet, A., 1925, *C.R. Soc. Biol.*, Paris, **93**, 219.

Dunkin, G. W., 1928, *J. Comp. Path.*, **41**, 94.

Edwards, J. T., 1926, *Agric. J. Ind.*, **21**, 6.

Erichsen, 1926, *Inaugural Dissertation*, Hannover.

Ernest, L. B., 1927, *J. Amer. Vet. Med. Ass.*, **71**, 742.

Holth, H., 1912, *Z. InfektKr.*, **11**, 378.

Hutrel, d'Arboval, 1826, *Dict. de Med. et de Chir. Vet.*, **1**, 516.

Reports of the International Veterinary Congress, 1909 & 1914.

Johne & Frothingham, 1895, *Deuts. Z. Tiermed.*, **21**, 438.

Male, G. P., 1911, *Vet. Journal*, **67**, 324.

Malm, O., 1911, *Norsk. Vet. Tdkr.*, **23**, 209, cited from *Deuts. tierärztl.Wschr.*, **20**, 564.

Markus, H., 1904, *Z. Tiermed.*, **8**, 68.

M'Fadyean, J., 1907, *J. Comp. Path.*, **20**, 48 ; 1918, *ibid.*, **31**, 73.

M'Fadyean, J. & Sheather, A. L., 1916, *J. Comp. Path.*, **29**, 62.

M'Fadyean, J., Sheather, A. L. & Edwards, J. T., 1912, *J. Comp. Path.*, **25**, 217 ; 1915, *ibid.*, **28**, 196 ; 1916[1], *ibid.*, **29**, 134 ; 1916[2], *ibid.*, **29**, 201.

Meyer, K. F., 1908, *Arb. Inst. InfektKr. Bern.*, **2**, 47 ; 1913, *J. Med. Res.*, **29**, 147.

Meyer, K. H., 1926, *Inaugural Dissertation*, Hannover.

Miessner, H., 1926, *Deuts. tierärztl. Wschr.*, **34**, 884.

Miessner, H. & Kohlstock, 1912, *Berl. tierärztl. Wschr.*, p. 450.

Miessner, H. & Trapp, 1910, *Mitt. K.-Wilh.-Inst. Landw.*, **2**, 219.

Plum, N., 1925, *K. VetHojsk Aarskr.*, p. 63.

Schalk, A. F., 1926, *194 Bull. N. Dak. Agric. Expt. Sta.*

Sheather, A. L., 1927, *J. Comp. Path.*, **40**, 224.

Turner, H. W., 1928, *J. Amer. Vet. Med. Ass.*, **72**, 1061.

Twort, C. C., 1914, *Vet. News*, **11**, 79.

Twort, C. C. & Craig, T., 1913, *Zbl. Bakt.*, Abt. I, Orig., **68**, 455.

Twort, F. W., 1910, *Proc. Roy. Soc.*, B., **83**, 156.

Twort, F. W. & Ingram, G. L. Y., 1913, *Johne's Disease* (Baillière, Tindall & Cox).

Vallée, H. & Rinjard, P., 1926, *Rev. gén. Méd. Vét.*, **35**, 1.

Wright, H. K., 1928, *J. Amer. Vet. Med. Ass.*, **72**, 1036.

CHAPTER VII. LEPROSY.

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History and Geographical Distribution.

HISTORY.

(W. BULLOCH).

THERE have been many different theories on the causation of leprosy, but it is now universally believed to be a specific infective disease caused by a bacillus (*B. lepræ*). This micro-organism was first described by the Norwegian leprologist, G. Armauer Hansen (1874). In a series of cases of leprosy he found, especially in the cells of freshly excised lepromata, fine rod-like structures, which he interpreted as bacilli. Some years later Hansen (1880) extended his observations by the utilization of staining methods, and also by attempted but unsuccessful inoculations in rabbits. In the same year the Breslau dermatologist, Albert Neisser (1879), who had visited Norway to study the disease, obtained a large amount of leprous material, which he utilized to great advantage. He gave a very exact account of the micro-organism which he found in enormous numbers in leprous lesions of the skin, cornea, testis, liver, spleen, peripheral nerves and lymph glands. From the constant presence of the organism in the lesions of leprosy and from the absence of other bacteria he came to the conclusion—and he was the first to do so—that leprosy is a specific disease due to a well-characterized organism, and that it must be infective. Neisser failed to grow the bacillus outside the body or to infect animals. In spite of very extended efforts by bacteriologists in all countries it appears to be still the fact that the bacillus has not been successfully cultivated *in vitro*, nor has the disease been conveyed experimentally to animals or even to man. Of considerable interest is the fact that a leprosy-like disease exists in rats (Stefansky, 1903; Lydia Rabinowitsch, 1903; and G. Dean, 1903).

W. B.

GEOGRAPHICAL DISTRIBUTION.

Leprosy, once a world-wide disease, is still endemic in countries where poverty and a low standard of living prevail. Rogers has shown the connection between the endemicity of leprosy and high humidity; this is undoubtedly true if large tracts are considered; but if smaller areas are examined, a high rate of incidence is found to be connected more closely with racial and tribal habits, famine conditions, a low standard of living, lack of sanitary laws and the prevalence of debilitating diseases ; in short, leprosy is most common where the general health of the people is low and where precautions against transmission are not taken.

It is said that in some parts of West Africa 1 in 16 of the inhabitants suffer, while in the Southern Soudan and other parts of Africa there is reason to believe that the disease is spreading rapidly. In South-East China leprosy is very prevalent, and the Chinese from these parts have carried the disease to South-East Asia and to various islands to which they have migrated. In India, though still very common, half a million cases not being an excessive estimate, leprosy appears on the whole to be stationary. In the West Indies and South America it is still very common. In the Balkan States and other countries of eastern Europe leprosy is still endemic.

Description of Organism.

GENERAL DESCRIPTION.

Leprosy is caused by the growth of *Bacillus lepræ* (*Mycobacterium lepræ*), commonly called the leprosy bacillus, in the body, and the reaction of the tissues to its presence. It consists of short rods, 2 to 4μ in length and about ·4μ in thickness. It resembles the tubercle bacillus in shape and staining properties, but is on the whole thicker, shorter and straighter, stains more easily with basic dyes, but is more easily decolorized. In dried smears stained by Ziehl-Neelsen's method it retains its colour for a considerable time. In sections mounted in neutral canada balsam it generally loses its colour within a few months, but mounted in euparal it remains bright red for more than a year. In this respect it resembles the mycobacterium of rat leprosy. The stain should be prepared with a 10 per cent. solution of fuchsine and applied cold. In dehydrating sections it is important to use as little alcohol as possible, otherwise the colour of some of the less stained bacilli may be destroyed.

In the quiescent phase of leprosy (see p. 366) the organisms are generally found to stain uniformly, but in the reactionary and resolution phases the staining is very irregular, sometimes the middle and sometimes the ends alone retaining the colour. Round, spore-like swellings, in diameter two or three times the thickness of the organism, and stained more deeply, appear either at the centre or at one or both ends. Thus a mass of bacilli often has the granular appearance mentioned by some writers. In certain types of lesions some organisms stain more deeply than others and some do not stain at all, their presence being indicated only by uncoloured spaces showing in the background ; in fact, because of this and also for other reasons mentioned on p. 359, the question arises whether there is not a non-acid-fast stage of the organism, which has not yet been shown up by staining.

Lepra cells. In the tissues *B. lepræ* is chiefly situated in round, epithelioid cells called lepra cells. The bacilli may be found in ones and twos, but in the more marked skin lesions they appear in masses, shaped like cigar bundles, suggesting that they multiply by lateral fission, each organism lying side by side with that from which it originated. A mucus-like substance called the glœa surrounds the bacilli; when their multiplication has caused the destruction of the containing cells, the glœa-bacillary mass of adjacent cells adheres together, moulded into strange shapes by the pressure of the surrounding tissues. Whether this glœa is excreted by the organisms or is formed by the cells is not known. Its composition has not been ascertained; it is not acid-fast.

CULTIVATION.

Various attempts were made to cultivate *B. lepræ in vitro* from 1885 to 1915. A detailed account of this work up to 1913 with references is given by Wolbach and Honeij (1914), to which readers are referred for the work of authors mentioned in the text but not included in the list of references at the end of the chapter.

The first attempts were made by Bordoni-Uffreduzzi, who succeeded in growing a diphtheroid on peptone, glycerin and blood-serum, which became acid-fast upon subculture. Similar organisms were obtained by Babes, Gianturco and others. In 1892 strictly acid-fast anaerobes were grown by Ducrey, Campana and others, who used peptone broth-agar with grape sugar. In 1901 Kedrowski obtained a diphtheroid which on subculture became acid-fast, by using a medium of placenta extract and agar. Bayon in 1911, using a similar medium, grew a like organism. Twort in 1910, following the analogy of his culture of Johne's bacillus on egg medium to which dried and powdered Timothy grass bacilli had been added, prepared a similar medium for the growth of *B. lepræ* by adding dried and powdered tubercle bacilli to Dorset's egg medium with glycerin and saline. He grew an unpigmented culture of acid-fast organisms. Bayon obtained a partly acid-fast growth, using dried smegma bacilli in horse-serum; this growth he injected into mice and thus got a pure acid-fast culture. Likewise Marchoux, using glycerin, potato and ascitic fluid, obtained a mixed growth which gave a pure acid-fast culture after injection into the peritoneal cavity of rats.

Following out the hypothesis that *B. lepræ* requires for its growth *in vitro* partly broken-down proteins, Clegg grew various cultures on a medium containing living amœbæ and cholera vibrios. Duval, using Clegg's medium, obtained similar growths, but later found that the amœbæ and vibrios were not necessary, as the symbiosis of other contaminating organisms had sufficient proteolytic effect to produce the culture; they found that the acid-fast organisms in such cultures remained alive, while other organisms were destroyed by exposure to a temperature of 60 to 62° C. for half an hour.

Another proteolytic method was that of Rost, Williams and others, who, following out the theory promulgated by Hutchinson that leprosy is caused by eating decomposed fish, tried to obtain growths on media containing various kinds of rotten fish. Their cultures were partly acid-fast.

Walker was able to obtain acid-fast bacilli from leprous lesions on Clegg's medium, but found that neither the symbiotic amœbæ of Clegg nor the protein-split products of Duval are necessary for the growth of this organism, the essential factor being a lean medium containing traces of nutrient substances.

The material from which cultures are obtained is important. The earlier workers used tissues obtained from lepers *post mortem*, while Clegg, Duval, Marchoux, Twort and others used nasal secretion. Some writers only mention that they used ' leprous material '. Others, again, obtained their cultures from aseptically excised nodules. The work of Fraser and Fletcher (1915) is important. They were able to grow diphtheroids at first, but afterwards, with improved technique, in which they took their material from the under surface of a carefully reflected skin flap containing numerous acid-fast organisms, they failed to get any result with the media used by earlier workers. Their experiments go far to prove that the cultures described above were due to contamination.

The cultures grown may be divided into non-acid-fast diphtheroids, stable acid-fast organisms, and others in an intermediate position. These last cease to be acid-fast when grown on agar, potato, &c., and become so again on media containing egg, &c. Some of the cultures are chromogenic and others white or greyish in appearance. A few of them show only slow growth, but most of them grow fast, either at first or after their first subculture. Taking into consideration the slow growth of the allied tubercle group and of *B. lepræ* in the human body, one would expect the *in vitro* culture of *B. lepræ* to develop slowly. An experience of the author's may be mentioned to explain the primarily slow and secondarily fast growth described by certain workers. A medium was prepared by growing smegma bacilli on Petroff's egg medium and sterilized by heating for half an hour to a temperature thought to be more than sufficient to destroy a smegma culture. Leprous skin containing large numbers of acid-fast organisms was prepared with the most careful precautions and implanted on this medium. All the tubes remained as they were for fifteen months, except that the fragments of tissue became deeply embedded in the medium; then a greyish cauliflower-like growth began to appear over the site of the tissue fragment in one tube and slowly increased to the size of a pea in the course of three months. Examination of this culture showed it to be acid-fast. Subcultures on Petroff's medium grew fairly rapidly. Subcultures on various media, with controls on similar media taken from a stock smegma bacillus culture, resulted in the growth gradually taking on all the morphological, staining and macroscopic appearances of the smegma bacillus. Thus slow initial growth may be due to cultural conditions, and when it is followed by incompatibly fast growth in a subculture some such fallacy should always be suspected.

Tests of Specificity.

The difficulty in testing cultures as to their being actually *B. lepræ* lies in the fact that so far it has not been found possible to produce by their inoculation or application in experimental animals a progressive disease resembling human leprosy. This is not to be wondered at seeing that the similar application of leprous material taken from patients has also failed. Bayon (1915) argues that ' any micro-organism isolated from lepers which claims to correspond to the acid-fast rod seen in lesions should be identified by injection into animals and the following production of the well-known histological features of lepromas. The Kedrowsky and those similar to this type are the only ones to fulfil this essential postulate '. But to those familiar with the lesions in the human tissues, Bayon's descriptions and beautifully coloured plates are not at all convincing. Duval and Harris (1913) after extended experiments state that ' animal experiment is not of use in distinguishing acid-fast bacteria, for the same lesions are induced by all, whether they be Timothy grass, milk, butter, smegma or the reputed leprosy bacilli '. Moreover, Fraser and Fletcher (1915), as also Bayon, have shown that acid-fast organisms which have been destroyed by heat, produce the same lesions as those that are living. Marchoux (1911), by injecting his partly acid-fast culture intraperitoneally into rats, obtained a pure acid-fast culture, the contaminating organisms being destroyed by the rats' tissues. Acid-fast bacilli have thus a power to resist animal tissues for a time and in so doing to produce a more or less definite histological picture, resembling that found in certain leprous lesions. Even if the bacilli of a supposed *B. lepræ* culture were to multiply within the tissues to a certain extent as they multiply *in vitro,* this would not justify our stating that a leprous lesion had been produced, nor would it be any proof that they were *B. lepræ.*

The other ground upon which the specificity of supposed leprosy organisms has been based is serological tests. Kritochewsky and Bierger and also Bayon claimed a specific complement-binding action for Kedrowsky's culture, which they failed to obtain with Duval's bacillus. But Duval and Harris (1913) state that ' the serum of animals immunized against repeated doses of Hansen's bacillus obtained directly from the uncontaminated human leprous nodule, reacts significantly with the slow-growing, acid-fast culture (Duval) and gives no specific reaction with any of the other so-called lepra cultures '.

There seems to be no doubt that the antigenic effect of any acid-fast organism depends largely on the medium on which it has been grown and on the method which has been used to prepare the antigen from the culture. Thus Taylor and Malone (1924) obtained complement fixation in leprosy with tubercle bacilli ' defatted ' by Dreyer's method ; and the author obtained even better results with a smegma bacillus culture which he prepared by drying and extracting with alcohol and then with ether till its weight was reduced by 50 per cent. It must be inferred, therefore, that serological tests are no more than group-specific and vary with different accidental and experimental factors. They have so far given no proof of the identity of any supposed culture of *B. lepræ*.

The question arises—if the growths of organisms obtained by various workers are not *B. lepræ*, what are they? There seems little doubt that the majority if not all of them are saprophytes which have contaminated the material taken or entered the culture medium later. Examination of the skin or nasal mucosa of healthy persons shows acid-fast organisms and others which, when grown on suitable media, tend to become partially or entirely acid-fast. In the ulcerating or encrusted nose of the leper the environment for their growth is even more favourable. They are also found on the surface of the skin and are able to persist there even after the ordinary means for cleansing and sterilizing it have been adopted. Like moulds they are also apt to cause contamination from the air unless very careful precautions are adopted. Material should always be taken by biopsy, as saprophytes are liable to increase in all the organs and tissues shortly after death, and it should be applied to the culture medium fresh and without delay.

ATTEMPTS TO PRODUCE LEPROSY IN EXPERIMENTAL ANIMALS.

These consist of (*a*) direct inoculation of leprous material into animals and (*b*) inoculation of various supposed cultures of *B. lepræ*.

(a) *Direct.*

Sugai and Duval both produced lesions in Japanese dancing mice. Nicolle and Blaizot and others, including the author, have produced lesions in *Macacus* monkeys; but similar lesions are produced by the subcutaneous injection of suspensions of leprous tissue which has been autoclaved at 120° C. for half an hour. Marchoux and Bourret (1909), stimulated by the results of Nicolle in lower monkeys, placed portions of a leprous nodule under the skin of the ear of a chimpanzee. Nodules developed which increased for three months, after which they began to diminish. This ape died three and a half months after inoculation. No nodules were found in liver or spleen. On section of the inoculated nodules there was centrally necrosed tissue with free bacilli and a few wandering cells; round this there was a mass of lymphocytes and mononuclears with more numerous bacilli inside the mononuclears; outside this there was organized connective tissue containing fixed cells with bacilli. The authors, however, considered that there was no evidence in this that an active lesion had been formed, as unpublished experiments of Borrel on abscesses produced by injection of dead tubercle bacilli gave similar pictures. They suggest that experiments with apes should be undertaken in the tropics, as it is difficult to keep them alive long enough to develop leprosy in the temperate zone. Experiments by Fraser and Fletcher with apes at Kuala Lumpur appear to have been negative, but more work is required along these lines, as it is to be expected that anthropoids, being more closely allied to man than other animals, should be more susceptible to leprosy. Also, seeing that leprosy occurs in man chiefly when his natural resistance has been lowered and when his diet is defective, attempts were made by the author to inoculate dethyroidized *Macacus* monkeys and monkeys fed on deficient food and on decomposed fish; these have so far failed to produce more than localized lesions, but similar experiments on apes might possibly be more successful.

Stanziale (1912) injected a suspension of leprous tissue in normal saline into the anterior chambers of the eyes of 12 rabbits, but obtained no growth. He also placed fragments of leprous tissue in the anterior chambers of 19 rabbits and apparently obtained a positive result with 8 of these. In both positives and negatives the tissue first increased in size, due to absorption of fluid, and then decreased for 30 to 60 days. Thereafter in the positive cases the fragment increased for 3 to 4 months to 7 or 8 times the size at inoculation. At the same time nodules developed on this mass or in its immediate neighbourhood. Microscopic examination of these showed that they were genuine granulomata and contained many acid-fast bacilli, which Stanziale considered were due to local multiplication. Repetition of this experiment is necessary. The question of the actual multiplication of bacilli is a difficult one as even a small fragment of fresh leprous tissue may contain a very large number of acid-fast organisms, and these, even when spread over a much larger mass and surrounding nodules, would still appear very numerous.

(b) *Indirect.*

Bayon (1915) considered that he had produced leprous lesions by inoculation of his own and Kedrowsky's cultures, whereas he failed to obtain such lesions with other supposed cultures of *B. lepræ*. Duval, by inoculating his culture in monkeys, produced lesions in parts of the body distant from the point of inoculation; no progressive disease, however, was noticed. Other workers also have inoculated their supposed cultures of *B. lepræ* with no more favourable results, failing to produce lesions any more characteristic of leprosy than may be obtained by the inoculation of such well-known saprophytes as Timothy grass, smegma and butter bacilli.

Discussion.

Reviewing the work that has been done so far on the cultivation of *B. lepræ in vitro* and on animal inoculation, the following points may be noted for guidance in future work:

(*a*) Material for culture should be taken in such a way that contamination by saprophytic and other organisms is excluded. Material taken in this way by Fraser and Fletcher from the under surface of a flap of leprous skin and with other due precautions failed to produce results with any of the media used by former workers, and the author has been equally unsuccessful after several years' trial. Various acid-fast and potentially acid-fast saprophytes are found on the surface of the body, in the nose and in the folds of the skin, as well as in the air, and these can only be excluded by very careful precautions.

(*b*) As pointed out by Fraser, it is due to the enormous numbers of acid-fast organisms in leprous material, that multiplication is often supposed to have occurred in culture tubes when smears are made, or in inoculated animals when sections are cut. Apparent macroscopic changes should be interpreted with care, as, when a fragment of leprous tissue is implanted on medium, the tissue cells may at first multiply or sink into the medium, giving an appearance which may be mistaken for culture growth.

(*c*) Serological tests of acid-fast cultures have not so far proved reliable.

(*d*) Living and dead acid-fast saprophytes produce lesions in inoculated animals very similar to those of leprosy. Unless, therefore, progressive disease is produced and repeated transmission from animal to animal is successful, this method cannot be considered a reliable test of the specificity of any suspected organism. In view of the fact that direct inoculation in animals of leprous tissue taken from all types and stages has proved negative, it is doubtful whether even a specific culture of *B. lepræ*, if it were available, would produce progressive disease.

(*e*) Considering the intracellular position of *B. lepræ*, perhaps the most promising medium is a culture of connective or other tissues of the human body. This was attempted by Fraser, as mentioned in his 1914 report, but he did not apparently meet with success.

(*f*) Attempts may also be made along the indirect line suggested by Zinsser and Carey, viz. that of first trying to grow *B. lepræ murium* (the causal organism of rat leprosy) on tissue cultures. While it is clear that there is no direct ætiological connection between these two mycobacteria, yet in many respects their characteristics are so analogous as to suggest that success in cultivating the one organism might show the path towards cultivating the other.

(*g*) The most promising lines of animal inoculation are by using anthropoid apes, either healthy animals or those the natural resistance of which has been artificially reduced. Also the anterior chamber of the eye, either of rabbits or of other animals, may prove a useful site for inoculation.

PERSISTENCE OUTSIDE MAN.

The only evidence of the vital persistence of *B. lepræ* outside the human body is afforded by a few scattered instances of patients who have developed lesions under circumstances which pointed to the infection having remained alive for some time on some instrument, fabric or other article. A few illustrations may be mentioned: (1) A highly infective cook worked in a household for some years with the result that his employer developed the disease; (2) a boy with bare knees played about in a house formerly inhabited by an infectious leper and from which all the furniture had been removed by the previous occupant, except the mats on the floors; the boy afterwards developed leprous lesions, but on his knees alone; (3) Hansen mentioned the case of a young man showing signs of infection one year after he had worn a pair of old drawers given him by a leper; (4) Ginders records a statement by a Maori chief that, when leprosy was formerly more prevalent, it was spread by giving the sleeping-mat or clothes of a leper to an enemy. The absence of positive cultural and inoculation findings, however, makes more accurate knowledge impossible.

The power of *B. lepræ* to persist inside the human body and the analogies of tuberculosis and rat leprosy (see page 379) help to give us data from which the degree of extracorporeal vital persistence can at least be guessed at.

Source of Infection.

The impossibility of obtaining positive results either *in vitro* or by animal experiments makes it unlikely that *B. lepræ* ever multiplies in nature outside the human body. While it is possible that it may remain alive for a considerable time outside the tissues, there seems to be much evidence that infection is as a rule due to prolonged and close contact with patients suffering from the types of leprosy in which *B. lepræ* are found in very large numbers (B^3 cases). In these cases acid-fast bacilli are shed from ulcerating nodules, abrasions of the skin, in the nasal discharge and in the droplets of saliva which are expelled from the mouth when patients shout, sing, cough or breathe violently. The presence of the organisms in droplets may be proved by placing a glass slide before the mouth of such a patient when coughing and then fixing and staining, when numbers of acid-fast bacilli may be found. While such patients are always potential sources of infection, they are even more so in the reactionary phase (see page 366). It is then that nodules ulcerate and discharge bacillus-laden pus and that the nasal discharge is more profuse. In this phase also *B. lepræ* are found in the fæces, doubtless having been swallowed with the saliva, and in the urine, having been carried in the blood-stream from breaking-down lepromata to the kidneys.

The most dangerous patients are often those, who without showing any marked outward signs of leprosy, discharge organisms from the nose or from ulcerating nodules under their clothes. In countries where the disease is endemic, like India, such patients are not at all uncommon. It should be noted that the majority of ulcers on the fingers and toes are trophic in nature and seldom contain infection. Of 250 leprous ulcers examined by the author at the Purulia leper asylum only 3 showed acid-fast organisms; the remaining 247 were trophic ulcers. It is remarkable that the public and even some physicians look upon these trophic ulcers as the chief source of danger and wish to isolate patients suffering from them, while not recognizing the true danger in the infectious cases mentioned above.

INSECT CARRIERS.

The possibility of transmission of leprosy by insects has been carefully considered by various workers. As shown by Marchoux and Bourret, the mechanical carriage of organisms on the feet of *house flies* is a not unlikely method of transmission. Evidence as to the infection of the gut of flies is conflicting; the India Commission of 1890–91 found all trials negative, but other investigators have found on an average 50 per cent. of infected flies.

Scabies is a common disease among lepers and the classes among which leprosy is common. The gut of *Acarus scabiei* found on lepers is seldom infected, but there is no doubt that auto-inoculation is favoured by the scratching induced by the presence of this parasite. The same is true of *lice* and *tics*. *Bed bugs* are also qualified to carry infection. Rogers and Muir (1925) summarize the investigations of eight workers who found on an average infected gut in 9·9 per cent. of bugs fed on lepers, but only 0·88 per cent. positive when the insects were collected from the beds of lepers.

Only 4 out of 631 *mosquitoes* caught on lepers by various workers were found positive.

Leboeuf (1912), after doing a considerable amount of work at this subject, reviewing his own work and that of others, considers that, though insects may help in the transmission of leprosy, the part they play is at most an insignificant one.

Pathology.

METHODS OF TRANSMISSION.

Post-Natal.

Up to the end of the eighteenth century the generally accepted theory was that leprosy is a disease which is spread by contagion. But in the nineteenth century, largely due to the teaching of the Norwegian authorities, Danielssen and Boeck, in their book published in 1848, the theory of heredity became popular. This was upheld by a special committee of the Royal College of Physicians of London in 1862. Even as late as 1891 the special India Leprosy Commission placed in their report: ‘ Though in a scientific classification of diseases leprosy must be regarded as contagious and also inoculable, yet the extent to which it is propagated by these means is exceedingly small ’. Hutchinson, at that time one of the greatest authorities on leprosy, held that leprosy was caused by the eating of decomposing or badly preserved fish; commenting upon the report of the India Commission in 1890-91, twenty years after the discovery of the specific organism by Hansen, he wrote: ‘I feel convinced that if leprosy be contagious at all, it depends but to an almost infinitesimal extent upon contagion for its spread’. Fortunately the Executive Committee, who appointed the India Commission, repudiated their views and declared in favour of contagion. All subsequent authorities and conferences have declared in favour of leprosy being a contagious disease and the International Conference which met at Strasbourg (in 1923) based all their resolutions upon this understanding: 'Il faut faire savoir aux populations que la lèpre est une maladie contagieuse'.

The question of intrauterine transmission is also a difficult one. Pineda has shown that infection of the placenta and cord is not infrequent and he has found post-natal infection in the child. Rodrigues reports 4 out of 15 placentas infected. Sugai and Mononobe (1913) state that they found *B. lepræ* in small numbers in the circulating blood of 10 out of 12 children born of leprous parents and in the placenta in 9 cases; and also that the specific organism was found in the blood of a new-born child whose mother was not leprous, but whose father was. They also write that, if lepra bacilli are injected intravenously into pregnant rabbits, they are found after 24 hours in the heart of the fœtus.

The unanimous opinion of all, however, seems to be that, with one or two possible exceptions, children who are separated from their leprous parents at birth and are thereafter kept free from all chance of infection, do not develop leprosy, and that for practical purposes leprosy may be regarded as due to post-natal transmission.

Portals of Entrance.

The possible portals of entrance are the nasal, buccal and pharyngeal mucosa, the skin, and the respiratory and gastro-intestinal tracts.

Stricker promulgated the theory that the *nasal mucosa* was the most frequent site of inoculation as he found bacilli in over 83 per cent. of nasal septa in lepers. Kitasato found numerous bacilli in the epithelial cells of the noses of 8 out of 68 healthy persons who were either the children of lepers or were living with them. On the other hand, Brinkerhoff and Moore (1909) made a nasal examination of 392 healthy persons in a highly endemic area and found only one infected; and in Culion Leper Colony Solis and Wade (1925) found that of 250 children living with leprous parents, no child showed primary nasal infection who did not at the same time show positive skin lesions. It is possible that in cold climates, where the body is more protected by clothes and nasal catarrh is more common, the nose is more liable to inoculation than in warmer countries where the skin is less protected and therefore more liable to abrasions through which infection might enter. The fact that acid-fast bacilli are not found in the nasal mucosa does not, however, preclude the possibility that inoculation has taken place through the mucous membrane of the nasal septum. In early skin lesions, smears and sections frequently fail to show any acid-fast organisms; it is also possible that early nasal lesions may fail to give positive bacteriological results when a scraping is examined. In favour of this may be mentioned the frequent history, noticed by the author, of dry rhinitis preceding more definite signs of leprosy.

There is little or no evidence for or against a primary infection of the *buccal* and *pharyngeal mucosa,* lesions of these parts not becoming evident until they are well established in the skin of the face.

The *skin* is the most frequent site of first-noticed lesions. One thousand and fifty histories of these were collected by the author from all parts of India. The face, ears and external or extensor surfaces of the limbs showed the largest number ; while the scalp, neck, flexor aspects of the limbs, the abdomen and especially the waist were comparatively exempt. It was noticed that lesions of the feet were more common in stony districts, but seldom occurred in alluvial districts without stones. It is not contended that these first-noticed lesions indicate the sites of inoculation and represented definite primary lesions comparable with those found in syphilis. In some cases they may have been primary, but in others some local injury may have located an infection pre-existing in the body.

The question of insect transmission has been mentioned above, and it is probable that the chief role of insects is not as vectors, though they may act as such to a certain extent, but as sources of irritation leading through their bites to auto-inoculation, the patients scratching in the organisms lying under their nails or on the surface of the body.

There is no positive evidence as to *respiratory infection.* Leprosy of the lungs does occur, but its infrequency and the fact that it only appears in cases in which the disease is well established in other parts of the body, are against this being a common portal of infection.

The *gastro-intestinal tract* does not show lesions, but this is no reason why it may not be a portal of entry. It has been shown that tubercle bacilli and even carbon atoms can be carried through the gastro-intestinal mucosa into the body and it is possible that lepra organisms may enter in the same way. In support of this may be mentioned the analogy of rat leprosy. Marchoux was able to infect rats with this disease by feeding them on rat leprosy material, and the author and Henderson have done the same.

The probability is that the nasal septum, the skin and the gastro-intestinal tract are all portals of entry, that the frequency with which each of these is implicated varies with different countries, customs and other circumstances, but that the relative frequency of each is a matter which is not at present known.

Factors influencing Transmission.

There are four factors which very markedly influence transmission :

(a) *The closeness of contact.* There is abundant evidence to show that while leprosy may be transmitted by occasional and casual proximity it is much more frequently associated with a house, room and most of all a bed infection. The joint family system in India, the common, family box bed in colder regions, sexual and sartorial promiscuousness, and the close contact of the child with its mother in the earlier years are all important factors in the spread of infection.

(b) *The length of contact.* The longer the contact the more are the chances of transmission taking place.

(c) *The infectiousness of the patient.* This is very important and is a factor that has not been sufficiently considered. It is in the nodular, skin type (B^2 and B^3) that the danger really lies.

(d) *The state of health and natural resistance* of the person who comes in contact. There is reason to believe that the healthy human body is not as a rule subject to leprous infection and that when the disease occurs it is due to lowering of resistance plus infection.

THE SPREAD OF LEPROSY IN THE BODY.

The position of *B. lepræ* in the body between its first entrance and the appearance of the first signs of the disease is as obscure as the method of entry. The analogy of rat leprosy would point to the lymph nodes as the most likely place of storage. Marchoux found the apices of the lungs and the lymph nodes alone affected in rats that had been fed on rat leprosy material, and the author and Henderson found acid-fast organisms only in the prevertebral nodes. But later evidence has been brought forward that, when large doses of iodide are given to contacts with lepers, the nerve-trunks and in some cases the lymph nodes showed swelling and tenderness, indicating clearly that they were the sites of infection ; that is, progressive disease was present in these parts, but not evident clinically until acted upon by iodide. It is possible that the corium of the skin also may form a reservoir without showing any clinical signs. The author has found histological evidence of leprous infection beyond the border of a leprous lesion at a point where there was no clinical evidence of disease. In other cases the only evidence of leprosy consisted of deep, subcutaneous nodules, which appeared on the administration of iodide and which contained *B. lepræ.*

The long persistence of leprosy with few or no clinical signs is shown by one case in which two anæsthetic patches, one inch in diameter, were all the evidence that had been seen for a period of 22 years ; on examination, however, a considerably thickened great auricular nerve was found. As in rat leprosy, it is probable that there is in some cases a slight degree of disease persisting, progressing and retrogressing without clinical signs sufficient to be noticed, and which only declares itself when the natural resistance of the patient falls below a certain level. There is good reason to believe that, as is known to be the case in tuberculosis, there are, in regions where the disease is common, many patients infected with leprosy in whom it never becomes clinically recognizable.

It is possible that *B. lepræ* may remain alive and latent in the body without multiplying for a certain length of time, but it is obviously impossible to distinguish this latent period, if it exists, from the incubation or preclinical progressive period. It has been calculated that the time between infection and the appearance of active clinical signs averages about 3 years, but it varies from a few weeks to over 20 years, and the length of the period varies with the intelligence and power of observation of the patient, who may fail to notice the earlier signs and recognize only some later exacerbation.

It is impossible to know with any degree of certainty how the germs are carried through the body from the site of entrance ; but the wandering cells of the reticulo-endothelial system may be suspected of being responsible. Later, when large lepra cells are formed and frequently break down, especially during the lepra reaction, the organisms are found in the blood-stream, which carries them, both free and contained in blood-cells, to various parts of the body. There is a general tendency whatever part of the body is attacked—skin, nerve-trunk or internal organ—for the neighbouring lymph nodes to become affected, but this is not always the case, and lymph nodes also frequently exhibit the disease when no trace of it can be found in the parts drained by their afferent vessels.

GENERAL PATHOLOGY.

The general result of infection with *B. lepræ* is the production of marked changes in the tissues of the body with proportionately little impairment of the general health. This is due to the local cytological proliferation and changes induced by the presence of the germs and the absence of any marked degree of toxicity.

While most of the tissues of the body may be invaded by *B. lepræ*, there are certain parts more affected than others and a few in which lesions practically never exist. The nerve-trunks and their branches, the skin and the nasal, buccal and pharyngeal mucosa show the most marked and important lesions. Of the internal organs the testis is perhaps most affected. The liver and spleen are also attacked, though their functions are seldom interfered with. No marked leprotic changes in the kidneys have been described, though in the Culion Leper Colony nephritis is one of the commonest causes of death. During reactions bacilluria occurs, but this appears to be due to transmission of the organisms through the kidneys from the blood and not to any lepromatous invasion of renal tissue. Lesions of the gastro-intestinal tract below the pharynx do not occur ; *B. lepræ* are found in the motions during reactions, but the source of these is the mouth, pharynx or nose ; it is possible that the organisms reach the blood-stream through the mucous membrane of the bowel or that they pass from the blood into the bowel, but of this no direct proof is available. The lungs are affected in a small proportion of cases ; large quantities of pus are coughed up, containing masses of acid-fast bacilli. In proportion to the numbers of these in the sputum the clinical symptoms are much less than would be expected in a case of tuberculosis of the lungs. The pancreas and suprarenals are apparently seldom or never attacked nor are the ovaries. The central nervous system is probably never directly invaded by leprosy.

Affections of the eyes are of great importance and these have been described later on page 367.

The scalp is particularly free from leprous lesions, probably on account of the firmness of its structure, the size of the hair follicles and the arrangement of the blood-vessels; but gross infection has been seen by the author in a few cases and anæsthesia of portions of the scalp is not uncommon, due to the involvement of its sensory nerve-trunks.

EVIDENCE OF DISEASE.

Evidence of disease is generally first available in the early stages from clinical signs before bacteriological proof is available; these are described later under practical diagnosis. Further evidence can be gathered from the reactions produced by potassium iodide, described on page 374. It is possible to learn much about pathological changes from post-mortem appearances, but these should be corrected by the examination of sections of tissues taken from living patients; for leprosy is seldom of itself a fatal disease and the complications from which patients die generally result in the alteration or destruction of the more typical histo-pathological appearances.

HISTOLOGY OF THE SKIN LESIONS.

Leprosy is generally classified among the infective granulomata.

(1) The examination of the earliest and mildest skin lesions shows an increase of the cellular elements in the most superficial, i.e. the papillary, layer of the corium, which underlies the basal layer of the epithelium. The epithelial ingrowths, viz. the hair follicles with their appendages the sebaceous glands, and the sudoriferous glands and ducts, are also coated by this increased cellular element, the disease thus being carried down deep into the corium. This cellular increase consists of the following elements: connective tissue cells; star-shaped mast cells containing in their cytoplasm granules which are to a great extent acid-fast when stained by the Ziehl-Neelsen method; large epithelioid cells apparently of endothelial origin, which, when they have ingested *B. lepræ*, are known as 'lepra cells'; and small cells similar in appearance to lymphocytes.

It is often impossible in such early lesions to discover any acid-fast bacilli. Whether this is due to their scarcity or to the infecting organisms not being acid-fast, though present, it is difficult to say. It is not likely that these cellular changes are due to the toxins of distant organisms and they must, therefore, be due to *B. lepræ* present locally. Nor are the changes caused by germs which were formerly present and have now died out, as the changes described occur in the active, extending margin of the lesion also. The presence of *B. lepræ* is also proved by the fact that, when the resistance of the patient is suddenly lowered by any intercurrent cause, such lesions often change into lesions of the type next to be described; and in the latter type organisms of various degrees of acid-fastness may be found, some retaining the stain dark red, others staining only slightly, and others appearing as ' ghost forms ', only their outline appearing in the counterstain.

In this earliest lesion there is a slight amount of flattening of the papillæ and in consequence of the interpapillary spaces of the epithelium. The infection does not appear to invade the epithelial elements, but it is accompanied by trophic changes in the epithelium, such as parakeratosis and hyperkeratosis. The hairs become affected and break off at the mouth of the follicle, the part inside the follicle becoming softened and swollen with a bulbous end. There is loss of the sweat function. Granulation appears to be perivascular and to follow and surround the vascular plexus which immediately underlies the epithelium, but there is often at first no vascular engorgement. Such lesions may remain stationary or may advance slowly, the infection probably being spread by mobile cells which ingest the organisms and carry them forward in the intercellular lymph spaces surrounding the superficial vascular plexus.

The nerve-branches supplying such a cutaneous lesion seem invariably to be affected, infection with cellular changes similar to those described in the skin gradually spreading upwards and affecting the larger branches and then the nerve-trunks. In the nerves the various connective tissue layers surrounding the axis-cylinders (endo-, peri- and epineurium) become the site of a granulation process similar to that described in the corium. The fibres themselves are, to start with, affected by the pressure of the cells. Apparently the non-medullated, sympathetic nerve-fibres are the first to suffer, and this, together with the cell proliferation, causes the changes in the epithelium—keratosis, loss of sweat function and changes in the hair follicles; while at the same time there is loss of epicritic sensation and of pigmentation. If the pressure is prolonged the fibres are destroyed; but, if resolution takes place in time, the fibres are preserved and function is restored. In other lesions the nerves appear to be affected first, and it is possible (though proof is wanting) that there may be a descending infection of the skin from the nerves.

(2) The lesion described above is of the most mild and chronic nature. When the resistance of the patient, either local or general, is less, the lesion is subacute, its margin spreads more rapidly and shows engorgement of blood-vessels. In this type a few acid-fast organisms can be found in sections; they lie chiefly inside the large epithelioid (lepra) cells, and there are also a few lying apparently in the intercellular spaces. In this lesion there are often multinuclear giant cells as described by Kedrowsky (1914), Bayon (1915) and Henderson (1928), very similar in appearance to the typical giant cells of Langhans found in tuberculosis. Here the granuloma

tends to become more thickened and to spread to the deeper vascular plexus. The centre of the lesion does not show engorgement of the vessels, but only the periphery.

(3) A third type of lesion rather more acute than the last may arise from either of the types described above or may belong to this type from the beginning. There is engorgement of the superficial capillary plexus throughout, but the invasion of the nerve-branches is less certain, being sometimes present and sometimes absent. Giant cells are not found as a rule, and epicritic anæsthesia, keratosis, anhydrosis and depilation may or may not occur. Acid-fast organisms are present in larger numbers, and granulation of the corium, though it does not yet invade the lowest layers, is more widespread. The epithelium is raised above the level of the surrounding skin surface by the pressure of the granulomatous corium and is flattened out and thinned, the interpapillary spaces disappearing.

(4) In the fourth type there is further progression from the last. The lower layers of the corium are invaded ; lepra cells are large, some containing large numbers of acid-fast bacilli and others few, while some organisms are to be seen in the intercellular spaces, possibly set free from broken-down cells. Pressure on the hair follicles and sweat organs causes their gradual destruction. The epithelium is forced outwards between the rows of hair follicles and the natural folds of the skin are exaggerated, giving the surface a tesselated or mosaic appearance. The most superficial layer of the corium is œdematous and comparatively free from acid-fast organisms, lepra cells and cellular infiltration. This is in marked contrast to the first type in which the superficial layer alone is affected. Such a lesion may originate either by a more superficial infection spreading to the deeper layers of the corium or by the extension of a focus deep in the corium which has been produced by a mycobacterial embolus in a vessel of the deep vascular plexus.

(5) The fifth type is the cutaneous nodule, starting round a deep embolus, but unlike the last it does not spread through the skin but forms a round circumscribed mass. In the centre there are aggregations of lepra cells, intermixed with small, round cells, while at the margin the proportion of the latter is greater. As a nodule becomes old the granular formation is often replaced to a large extent by fibrous tissue making it firm and hard. During severe reactions a nodule frequently shows degenerative changes and this may go on to pus formation, the pus being absorbed or discharged through a small perforation in the epithelium, or resulting in destruction of epithelium and the production of an ulcer of which the remains of the nodule forms the hard base. At other times nodules are resolved, leaving only a small quantity of fibrous tissue under the thin epithelium.

These various types of skin and subcutaneous lesions have been described, but one type often merges into another, and lowering of resistance generally tends to change a less into a more severe type.

LESIONS OF THE NERVES.

Equally important with lesions of the skin are those of the nerves. As has been mentioned, the central nervous system is seldom or never affected, but any of the peripheral nerves and their branches may become involved. Disease is found most frequently in subcutaneous nerves, especially in those most liable to injury, torsion, bending or pressure; thus the commonest nerve-trunks to be affected are the ulnar, peroneal and great auricular.

The histo-pathology of nerve-lesions corresponds more or less closely with that of the first three types of skin lesions described above. The nerve-fibres are destroyed by pressure of the granuloma, which infiltrates the connective tissue elements of the nerve, and later by the contraction of the new fibrous tissue which replaces the granular formation.

Nerve-trunks are invaded either by ascending lesions from the skin or by metastatic embolism. Proof of ascending invasion is exemplified when from a cutaneous lesion of the forearm thickening of branches of the radial and ulnar nerves gradually proceeds upwards to the respective trunks; proof of metastatic infection is shown when nerve-trunks are found affected but their branches and the skin supplied by them remain free.

One of the most important phenomena in leprosy has been referred to above, namely that the milder the type of lesion and the greater the resistance of the patient the more are the nerves involved; and inversely, the grosser the lesion and the lower the resistance the less is the disease found in the nerves. This cannot be explained by any essential difference either in the patient or in the strain of organism, for in the majority of patients the disease begins with the nerve type, passing on later into the skin type, and patients in whom the former has been maintained for years will pass on rapidly into the latter if their resistance is markedly lowered by an intercurrent disease or by any other cause; and patients who are suffering from the grosser skin types will show a marked increase of nerve involvement as the skin lesions diminish either naturally or under treatment. Many theories have been put forward to explain this phenomenon. Perhaps the most likely hypothesis is that *B. lepræ*, when the resistance is low, quickly multiplies inside the ingesting cells and immobilization takes place; whereas when the resistance is high the bacilli are able to travel up the nerve-branches from the skin, by direct spread, possibly of a non-acid-fast or less acid-fast form.

While in early leprosy the small cutaneous branches are most affected, in the last stage of the disease the trunks show most signs. In that stage, therefore, whether the disease has been chronic throughout and never developed the grosser skin type, or whether in the intermediate stage there may have been gross skin lesions, we have the well-known acroteric (glove and stocking) trophic lesions due to pressure exerted on the axis-cylinders, first by the cell proliferation and later by the contraction of fibrous tissue. Seeing that the longer the nerve-trunk is the greater is the length of nerve-fibre over which pressure is applied, the distal parts of the limbs, i.e. the hands and feet, show the first and most marked signs of trophic involvement. Thus there is anæsthesia, both epicritic and protopathic, gradually spreading up the limbs from the fingers and toes; thermal and pain sensations are lost; loss of sensation is often preceded by hyperæsthesia and paræsthesia. Side by side with these there are anhydrosis, depilation and changes in the nails; wasting and fibrosis first of the small muscles of the hands and feet and later of the forearms and legs. As the peroneal and ulnar nerves are most apt to be involved, the muscles supplied by these nerves are most markedly affected. Fibrosis of the small muscles of the hands and feet is followed by the typical claw hand (main en griffe) and the club-shaped foot; while drop-foot is a common result of wasting of the peroneal muscles. The bones are decalcified and slowly absorbed. Secondary infection often leads to abscesses and ulcers, while, apart from secondary infection, blisters, blebs and perforating ulcers occur generally as the result of a reaction in the affected nerve-trunk.

Not only are the small muscles of the limbs affected, but anæsthesia of the face is often followed by fibrosis and contraction of the muscles of expression, especially the orbicularis oculi and orbicularis oris, giving the well-known facial expression of chronic nerve leprosy in contrast to the thickened, corrugated face (leontiasis) typical of the skin type.

CLASSIFICATION.

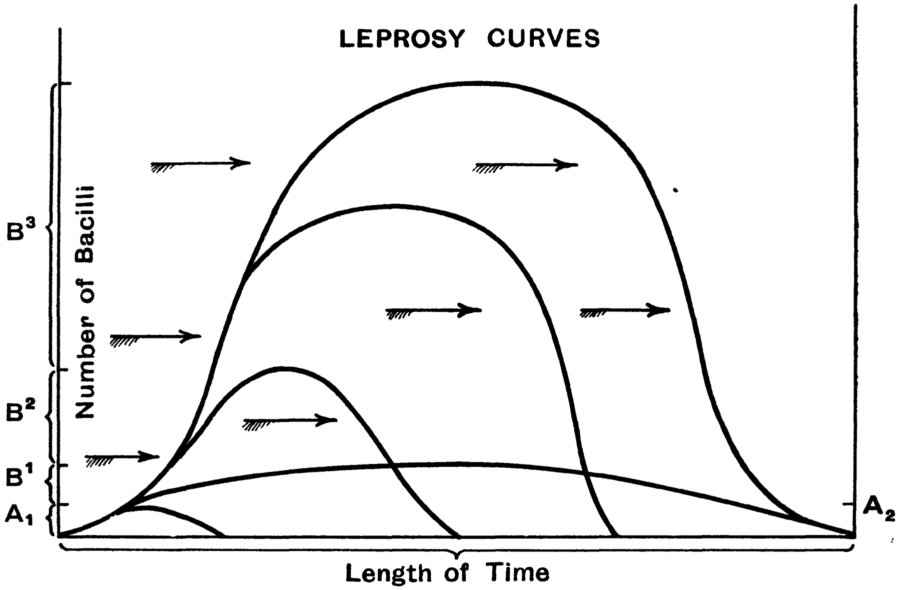
The old nomenclature of Danielsen which, slightly modified by Hansen (1895), divides leprosy into *tuberculosa*, or nodular, and maculo-anæsthetic types, with a mixed form combining the first two, is sufficient when only advanced cases are to be dealt with; but when the disease is studied in all its forms from beginning to end, and when it is realized that one type merges into another and that many of the worst skin cases do not show any nodules, having only diffuse cutaneous lesions, it is clear that some more scientific and comprehensive classification is necessary.

The classification described below has been found by the author a practical and useful one. The principles upon which it is founded are as follows:

(*a*) Leprosy is a self-healing disease increasing up to a certain point and then tending to die out, although twenty or more years may be required to complete its entire course. Many patients die of complications, but it is seldom of itself a fatal disease.

(*b*) Speaking generally there is an inverse ratio between the resistance of the patient and the multiplication of bacilli in the body.

(*c*) The more the bacilli the less the involvement of the nerves, and vice versa the fewer the bacilli the greater the involvement of the nerves.



A = Nerve (anaesthetic) leprosy
A1 = Primary nerve leprosy.
A2 = Secondary nerve leprosy.
-> = Reaction-producing causes.

B = Skin (nodular) leprosy
B1 = First stage of ditto—few bacilli found in the skin
B2 = Second stage of ditto-more bacilli found in the skin.
B3 = Third stage of ditto-very marked. generalised lepromatous infiltration, abundant bacilli

The type and stage of the disease is indicated by tracing curves as in the figure. The perpendicular indicates the increase of *B. lepræ* and the horizontal shows time. The more rapid the increase of leprosy organisms the more perpendicular the curve. In a typical case of skin-leprosy the curve rises to a maximum and then falls as the disease begins to diminish and the organisms die out. The greater the resistance the flatter the curve. An imaginary line drawn parallel to the base line divides the field into two parts, A and B, and indicates the point in the course of the disease at which *B. lepræ* can be found on bacteriological examination of the skin, mucosa or lymph nodes. All curves begin below this line, i.e. in all cases the number of organisms is originally so few that they cannot be found microscopically; some curves remain flat and below the line, i.e. those which are throughout purely of the neural type with relatively high resistance and in which bacteriological examinations remain negative, diagnosis depending entirely upon clinical signs. In other cases the curve rises above the line into the B area, more or less rapidly and to a greater or less height according as there is more or less resistance. At any point the curve, whether high or flat, may alter its direction; thus a flat curve may suddenly rise into the B area if the patient's resistance is lowered by some intercurrent cause, indicating that the *B. lepræ* have increased to an extent that they can be discovered microscopically; on the other hand a rapidly rising curve may be flattened out or begin to fall, indicating that treatment or the removal of some resistance-depressing condition has caused the disease to recede and the number of *B. lepræ* to diminish. For convenience the B area is divided into B^1, B^2 and B^3, the first of these including cases in which only a few acid-fast organisms can be found, the third those in which very large numbers of organisms are present, and B^2 intermediate between B^1 and B^3. The A area also is divided, but in a different way, viz. according to the nature of the nerve-lesions. In early cases, as mentioned above, the lesions take the form chiefly of superficially anæsthetic patches, the terminal sensory branches of the skin being affected; in the later stages the lesions are mainly due to blocking of nerve-trunks and the signs are, therefore, acroteric, trophic disturbances. The former are represented by the beginning of the curve and are called A_1; the latter, placed at the end of the curve, are called A_2.

There is necessarily no clear line of demarcation between the various types and stages of leprosy. Often the disease is found passing from one stage to another, either increasing and passing up the curve or decreasing and passing down the curve. If there is increase and it is doubtful whether a case should be classified as B^1 or B^2 the formula B^1–B^2 is used and likewise B^2–B^3 on the same principle. In a case in which the disease is diminishing, B^3–B^2 or B^2–B^1 may be used. Sometimes the trophic A_2 signs may be marked in the limbs while in the same patient B^3 signs are still distinct, though diminishing, in the face and ears; such a case is classified as B^3–A_2. Likewise in purely nerve cases, when A_1 and A_2 signs are present side by side, A_1–A_2 is a useful denomination. Much of the vagueness and apparent contradiction which characterize the descriptions of lesions in the works of earlier writers on leprosy is due to the lack of some clear classification based on a study of the course of the disease and the interrelationship of the various complicated types and stages. The above classification aims at clearing up this confusion and placing our knowledge of leprosy, as it gradually accumulates, in a clear and orderly form.

The Reaction in Leprosy.

B. lepræ differs from most ordinary bacillary and coccal infections in its low toxicity, the lipoid material in its composition, its intracellular growth and the presence of a mucoid substance which accumulates round about it even after the cell has been destroyed. In the typical leproma, such as we find in B^2 and B^3 cases, we have, therefore, a threefold protective mechanism consisting of the composition of the bacilli, the lipoid material tending to prevent its destruction; its intracellular position, the lepra cell acting more as a host than as a phagocyte; and the mucoid material, called by the earlier writers the 'glœa'. This protective mechanism enables the organism to multiply enormously, forming masses within separate cells, which, as the cells become further expanded and destroyed, coalesce, the bacilli embedded in the glœa being moulded in the intercellular spaces by the pressure of the surrounding cells.

The process of multiplication of bacilli may go on for months or years quietly without producing any marked local or constitutional disturbance. This period we may call the *quiescent phase.* When, however, due to any cause, there is a sudden breaking up of the lepra cells or liquefaction of the glœa, the bacilli are brought into contact with the surrounding tissues and a reaction takes place ; there is engorgement of the blood-vessels, dilatation of the perivascular lymph spaces and marked local diapedesis of leucocytes, especially polymorphs, which phagocytose the organisms. Clinically there is sudden swelling and erythema of the affected lesions, and the appearance of rose-coloured cutaneous nodules in different parts of the body. As systemic changes we find a rise of temperature, bacillæmia and alterations in the blood-serum indicated by acceleration of erythrocyte sedimentation (Muir, 1928[2]). Only the lepra cells which are ripe and the glœa masses take part in the reaction, and thus lesions which do not contain ripe cells do not react. For this reason reactions are comparatively uncommon in cases of the A types, though even in them reactions do occur in the nerve-trunks. Sections of a reacting lesion may show in the deeper layers of the corium vascular engorgement, perivascular œdema, marked polymorph leucocytosis with disappearance of acid-fast organisms; while the more superficial layers are still quiescent, with the bacilli chiefly intracellular but contained in the cells only in small numbers ; in other words the deep parts having ripe cells have reacted in contrast to the superficial parts which remained quiescent, the lepra cells being immature.

Causes of Reaction.

The causes which bring about the reactionary phase spontaneously appear to be many ; among them may be mentioned febrile intercurrent diseases, gastro-intestinal disturbances and unaccustomed physical exertion. It may also be caused therapeutically by the injection of certain drugs such as hydnocarpus oil and its preparations, and it has been noted after vaccination for smallpox. Potassium iodide has a specific effect in this direction ; in certain cases in which ripe lepra cells or glœa masses are abundant as little as 1 or 2 grains given orally will produce a very marked reaction.

Effects of Reaction.

There are four well-marked effects resulting from reaction :

(*a*) Resolution is brought about in the area which has reacted.

(*b*) Bacilli are set free in the blood-stream with resulting metastasis.

(*c*) There is a temporary depressant action on the general resistance of the body.

(*d*) When wisely induced with therapeutic intent by drugs in suitable doses, and at proper intervals, immunity results ; contrariwise, when reactions occur accidentally or are induced without safe precautions, an allergic condition is liable to result which is detrimental to the patient. This allergic condition is what is commonly known as lepra fever ; the febrile condition may last for several weeks or even months, repeated reactions being induced and maintained by the high temperature and by the weak condition of the patient. One of the signs of immunity is the disappearance after two or three days of the rose-coloured, cutaneous nodules of embolic origin ; in progressive cases, in which immunity has not yet become sufficient, these nodules form the nuclei of new, spreading lesions.

The term *phase* has been used because of the repeated recurrence of reactions. We have thus three phases: the *quiescent,* the *reactionary* and the *resolutionary*. The signs of resolution are indicated histologically by the appearance of resistant forms, spore-like swellings in the mycobacteria, granulation or loss of acid-fast staining, disappearance of acid-fasts and lepra cells, the cellular granuloma being replaced by fibrous tissue.

THE MUCOUS MEMBRANES.

The nasal septum is one of the parts most affected in leprosy, perforation of the cartilage being common ; in contrast to syphilis the bones are not affected. The accessory sinuses are seldom involved. The mucous membrane of the tongue is affected in many B^3 cases, showing a corrugated and sometimes even a nodular appearance. The hard palate may be the site of leprous infiltration but seldom shows any swelling; when perforation occurs it may be taken as an indication that accompanying syphilis is present. The soft palate, however, is often profoundly affected, showing ulceration which may lead to almost complete disappearance. Leprous infiltration of the glottis sometimes leads to asphyxia, which may have to be relieved by tracheotomy. The tonsils are not notably diseased.

THE EYES.

Hansen and Looft (1895) state that ' the eyes are, in the nodular form, almost always affected '. The author's experience in India differs from this even if the word ' nodular ' be taken to mean only B^3 cases, as even in this type he has found signs in less than 50 per cent. and in the milder B types it is even less common.

The eye lesions of leprosy should be divided into two classes: the direct, in which there is bacillary invasion of the organ; and the indirect, in which involvement is due to sensory and trophic changes in the cornea and the ocular appendages.

Direct Involvement.

The eye, as is the case in other regions of the body, is invaded either by direct spread from the surrounding skin or by organisms being conveyed in the blood-stream from other parts of the body. The former is undoubtedly the commoner of the two, as direct lesions seldom occur if the surrounding skin is not markedly affected. In the milder cases, conjunctival or subconjunctival invasion may take place with but few signs of swelling, and the upper part of the cornea may become gradually infiltrated with the disease, giving an appearance like ground glass. In the more severe type there is nodule formation at the outer part of the limbus, which may spread superficially over the cornea, projecting from its surface like an exaggerated pterygium; or it may project from the inner surface of the cornea into the anterior chamber; or it may be intermediate in position. The iris also is liable to be attacked, showing either small nodules on its surface, or more commonly a general infiltration, causing immobilization or obliteration of the pupil. In all these subtypes complete blindness is liable to occur, especially if the eye is involved in a reaction.

Indirect Involvement.

In the A_2 type anæsthesia of the cornea and wasting and relaxation of the orbicularis oculi remove the protective mechanism of the cornea, which is in consequence liable to become inflamed or ulcerated.

THE LYMPHATIC SYSTEM.

The lymphatic system holds a very important place in leprosy. There is evidence to show that the lymph nodes act as reservoirs of *B. lepræ* in cases in which signs of the disease have not been observed. The lymph nodes most affected are the subinguinals and to a less extent those of the epitrochlear and axillary groups; the cervical and supraclavicular groups are less affected. The iliac glands are also affected. When the liver and spleen are the seat of disease the lymph nodes of the hila of these organs are infiltrated. Many nodes which clinically do not appear to be swollen, swell up when potassium iodide is administered. The infection tends to linger on in the lymph nodes after it has cleared up in other parts of the body.

As a rule the nodes retain their natural shape, becoming swollen as a whole, and do not become globular as they do in tuberculosis. On section the yellowish colour of the ampullæ and medullary cords is characteristic. Sometimes only a few ampullæ are diseased, and these stand out macroscopically from the healthy part of the gland. Microscopically lepra cells full of acid-fasts occupy the centre of the ampulla, the small round cells tending to range themselves round these. The cortical sinuses are congested and there may be marked fibrous thickening of the capsule. Hansen and Looft (1895) point out the filter action of the lymph nodes.

THE LIVER AND SPLEEN.

These are probably more often affected than post-mortem appearances would lead one to suspect; for in B^3 cases the administration of potassium iodide sometimes leads to a marked reaction in these organs, which become considerably enlarged. In the liver, on histological examination the disease is found to follow the portal canals, but the parenchymatous cells are not invaded by the organisms, though the multiplication of lepra cells causes atrophy and destruction of liver cells through pressure. The organisms lie in the endothelial cells, which become enlarged and form lepra cells. In the spleen the infection lies in the walls of the arterial capillaries and Malpighian bodies.

THE TESTES.

Leprosy of the testicles is of importance, as in most cases of the B^2 and B^3 types they are affected. Even when there are no clinical signs, iodide will cause severe reaction with swelling and engorgement, which subsides again in a few days, recurring with each successive dose till the disease has cleared up. It may be noted that, whereas the parenchymatous cells in other organs are not invaded by the bacilli, only the endothelial cells of the blood-vessels and the stroma being affected, in the testes the epithelial cells of the seminiferous tubules are apparently also invaded; this is probably due to the nature of their embryonic origin. The intracellular position of leprosy organisms was established by Hansen (1895) (in opposition to Unna's [1896] view that they lie in the intercellular lymph spaces and are never intracellular) by their position in the testes, being 'chiefly in the lumen of the seminal canals, where no lymph vessels exist'.

The ovaries and female genital tract are not affected by leprosy as a rule.

The lungs, pancreas, kidneys, gastro-intestinal tract and central nervous system have already been referred to.

THE CIRCULATORY SYSTEM.

The circulatory system is not as a whole markedly affected in leprosy. The heart is not invaded and no characteristic change in the circulation has been noted apart from what occurs in other debilitating diseases. In affected organs the disease tends to follow the capillaries and there is strong evidence that lepra cells are at least as a rule of capillary endothelial origin. Wade (1927) states that 'arteriosclerosis has been observed as an unexpected feature of the picture in autopsies at Culion. This is not of the aorta but of the smaller vessels, and occurs chiefly as a medial degeneration. This has twice been seen in vessels of boys as young as 11 or 12 years who had died after prolonged lepra reaction'.

MUSCLES.

Direct, specific disease of the muscles does not occur in leprosy, even the stroma being unaffected as a rule. This may be contrasted with rat leprosy, in which the perimysium is directly invaded by the disease, the muscle fibres being destroyed by the pressure of lepra cells. The muscles are affected, however, by interference with their neurotrophic supply and the fibres degenerate into fibrous tissue, which again contracts like all newly formed tissue. As has been mentioned above, trophic changes occur chiefly in the A_2 type or stage of leprosy, and the muscles most affected are the small muscles of the hands and feet and those of the face.

THE BONES AND JOINTS.

The bones and joints are not, according to most authors, directly invaded by *B. lepræ*, but neural disease leads to very marked trophic changes in them. The small bones of the hands and feet naturally suffer most. Radiographs of affected parts show less dense shadows due to decalcification. The bones become gradually thinner and shorter until they are entirely absorbed. The phalanges suffer most, but the carpals, metacarpals, tarsals and metatarsals may also become gradually absorbed. In one case the author found that the talus had entirely disappeared, and the ends of the tibia and fibula, whittled down to sharp points, were placed in a hole in the dorsal surface of the calcaneus. Not infrequently sudden nerve reactions and the presence of secondary infection lead to necrosis ; this is described below in the section on *trophic ulcer.*

Trophic changes also take place in joints ; synovitis is not uncommon, especially in the ankle and knee, and the author has noticed bursitis in some cases. The ends of the bones may become thickened, giving a condition similar to that found in Charcot's disease.

A very common phenomenon, requiring further investigation as to its cause, is the extreme pain in some of the bones which occurs during the lepra reaction.

TROPHIC ULCER AND ALLIED CONDITIONS.

These phenomena are characteristic of leprosy. They occur when a reaction takes place in a nerve-trunk and are confined to the tissues which gather their trophic supply from the reacting nerve. The depth and severity of the lesion vary with the amount of the leproma in the nerve-trunk which is reacting and with the severity of the reaction. The slightest and most superficial is a blister and, when deeper, a bleb. In severer cases the whole thickness of the skin is involved, and in the most serious cases the deeper tissues including the bone. These lesions occur most commonly on the hands and feet ; when there is destruction of the skin of the sole of the foot, the term *perforating ulcer* is used.

SEROLOGICAL CHANGES.

The changes in the blood-serum in leprosy have been studied to a limited extent, but more investigation is necessary. The acceleration of the sedimentation of erythrocytes has been referred to elsewhere. In B^3 cases gelification and white precipitation occurs as in kala-azar on adding formaldehyde, but generally to a less extent than in that disease. These and other tests show that there is a marked change in the globulin content. Complement fixation and precipitation tests, using group-specific cultures as antigens, have been referred to on pp. 349 and 375.

One of the most striking features of leprosy is its strict limitation to man, and the failure of attempts to transmit the disease to experimental animals. In this it resembles rat leprosy, which appears to be limited to rats except that it may be inoculable to a limited extent in white mice, and, as Marchoux (1925) has shown, may infect human beings. Because of this and other features of resemblance the organisms of human and rat leprosy may be grouped together and distinguished from the tubercular group of pathogenic acid-fast organisms, which are so easily transmitted to other kinds of animals.

Resistance.

Leprosy is uncommon in the healthy aboriginal, when he lives in the jungles of India as a child of nature; indeed, he seems to enjoy to a limited extent the same resistance as the wild animals he lives amongst. When, however, the aboriginal comes in contact with the outside world and changes his more primitive life, mixing with people who have other habits and customs and leaving off his simple, though healthy, tribal rules of hygiene, he quickly tends to fall a victim to leprosy.

On the other hand, human communities in a more advanced stage of civilization appear to be once more beyond the reach of leprosy. In the Middle Ages leprosy was common in England, but some three or four hundred years ago it ceased to be endemic.

It has been put forward by certain writers that the relative freedom from leprosy of certain countries is due to an acquired racial immunity; but there is no reason to believe that this is so. Europeans living in eastern countries where the disease is endemic are just as liable to it as the native inhabitants, and there are many instances of Europeans who have contracted it from native servants and others, even in spite of their comparatively hygienic mode of living.

Wade and Rodrigues (1927), commenting on the apparently less serious form that leprosy shows in India as compared with the Philippines, suggested that the difference was due to a racial immunity acquired by the natives of India through its more prolonged prevalence in that country than in the Philippines. Study of leprosy in India, however, does not confirm this view. The milder nerve type is more common in the high and comparatively healthy plateau of the Deccan, whereas the more severe types are found in the lower and moister plains of Bengal and Bihar; but there is no reason to think that this is because leprosy has been prevalent for a longer time in the one part than in the other.

In fact, occurrence of the milder form rather than the graver, and exemption rather than prevalence, are due not to racial immunity or even to early, frequent slight infections in childhood (as appears to be the case in tuberculosis), but to the extent to which the general resistance of the body is lowered or broken down by various predisposing causes, which render the tissues of the body a suitable soil for the growth of *B. lepræ*. If a patient is attacked by malaria, influenza or some other disease which temporarily lowers his resistance still further, leprosy becomes established in the tissues to such a degree that it is of itself able to keep the resistance permanently lowered. For there is little doubt that, beyond the first stage of leprosy, there are changes in the blood-serum which correspond with changes in other diseases in which we find the resistance to leprosy lowered. For instance, in the B^2 and B^3 stages, the sedimentation of erythrocytes is permanently accelerated, and other diseases in which the same acceleration is found lead to an increase of the leprous process in the body. On the other hand, conditions in which sedimentation is retarded generally correspond with amelioration of that process.

When leprosy, therefore, has passed the first stage, it of itself lowers the natural resistance of the body, and thereby establishes the conditions necessary for its further development. In the third stage (B^3), however, another factor begins to show itself which corresponds to acquired immunity. The lepra cells have increased in number and in size, and, as they break down singly or in groups, set free antigens, which again give rise to antibodies. The degree of immunity thus produced may reach temporarily a high level, especially if for any reason a massive breaking up of lepra cells occurs, as it does in a reaction. This high degree of immunity may have a very marked effect in clearing up the lepromatous condition, and may cause rapid improvement of the condition of the patient, and, as will be shown in a subsequent section, this is taken advantage of in treatment.

In contrast with this immunity, an allergic condition may occur in which the resistance of the patient is lowered and leprosy makes rapid progress. The reason for this has been mentioned in other sections.

It may be stated generally that the more severe the type of leprosy a patient has suffered from, the greater the immunity he has acquired, and the less the likelihood is there of the disease ever recurring.

Age is an important factor in resistance ; in early infancy there would appear to be some special protection, as infants removed from infectious parents at birth practically never develop leprosy, although as Rodrigues (1926) and others have shown there may be an intra-uterine infection of the fœtus. Shortly after birth, however, and right on during the period of growth, children are much more liable to acquire infection and develop the disease than adults. Thus in the Culion colony 33 to 44 per cent. of children not separated from their infectious parents show signs of leprosy, whereas the percentage of conjugal infections averages only 2 to 5. While this may be due largely to the closer contact between parents and small children and the unconscious carelessness of the latter, there is also the possibility that the greater frequency of infection in childhood is due to lower natural resistance.

Diagnosis.

GENERAL.

It will be evident from the section on pathology that the practical diagnosis of leprosy can be made clinically in almost every case long before a positive bacteriological or serological result is obtainable. The reasons why diagnosis of leprosy is frequently delayed until the bacteriological examination is positive are the slow, insidious and painless onset of the disease and want of familiarity with its early signs. In certain purely neural cases (A_1 and A_2) the diagnosis must depend from beginning to end on clinical signs alone; in such cases acid-fast organisms might be found on nerve section, but such a procedure would as a rule be unjustifiable, as the thickening of nerve-trunks and the sensory and trophic changes leave no doubt as to the nature of the disease.

So also in the majority of less resistant cases clearly diagnostic clinical signs are almost always present for a considerable time before *B. lepræ* can be found by microscopic examination.

The importance of early clinical diagnosis of leprosy cannot be exaggerated, seeing that it is much more remediable and is not (there is good reason to believe) transmissible in the earliest stage.

It is beyond the scope of this work to give in detail the signs upon which clinical diagnosis is based. Loss of epicritic sensation with keratosis, anhydrosis, depigmentation (in dark skins) of a patch of skin, with marked thickening of the nerve supplying the part, may be considered as absolutely diagnostic. Sections of such a patch will not as a rule show any acid-fast organisms, but will show the cell infiltration in the papillary layer, described on p. 359, as occurring in the first type of lesion. If, however, there is a raised, erythematous margin, in which epicritic sensation is still present, sections or smears taken from this margin may show a few bacilli. It must be remembered that in skin lesions in which epicritic sensation is absent they are not found as a rule.

In cases in which organisms are found, deep, protopathic sensation is generally absent to a greater or less degree; the deeper the lesion and the greater the number of organisms found, the more pronounced is the protopathic anæsthesia. In these lesions thickening of nerves is absent or at least not marked.

Macular patches, by spreading radially may coalesce and thus cover large areas of the trunk, face or limbs. These areas are depigmented, and more or less of the nature of a scar, fibrous tissue having taken the place of the cellular infiltration; sensation is generally absent.

When no definite macules or patches or thickened nerves can be detected, but the skin shows suspicious thickening or erythema, especially about the face, a bacteriological examination of the lower part of the pinna should be made, as this is the region most commonly affected by leprosy.

Bacteriological Examination.

As the epithelium is not invaded by the organisms, a scraping taken from the surface is useless for bacteriological examination. If nodules are either visible on inspection or can be detected by pressing the pinna between the finger and thumb, or if there is considerable diffuse swelling, it is sufficient to make an incision with a sharp scalpel and scrape the sides of the incision with the blade, and then smear the material so gathered on to a slide. On drying, fixing by heat and staining by Ziehl-Neelsen's method, large numbers of acid-fast organisms may be found. If they are not found, or if it is suspected from the beginning that they are few in number, it is advisable to cut off from the edge of the ear by means of a pair of scissors curved on the flat, a small piece of skin which should be at least 1 mm. in thickness; this should be applied, raw surface downwards, to a slide and pressed with the scissors on to the glass until all the soft material is smeared on the slide, only the epithelium remaining. By this method any organisms that may be in the tissues are expressed. Where doubt still remains, however, it is always wise to cut sections and examine for the characteristic cell infiltration lining the basal layer of epithelium.

Another site of importance is the nasal septum. This should be inspected either by pressing the tip of the nose upwards and backwards, or with the aid of a nasal speculum. Leprosy may be suspected if the mucous membrane has not the normal, bright red, moist appearance. In A_1 cases there is merely dryness and a less deep red colour, bacteriological examination being negative. In cases of the B type there may be ulceration, generally covered with a dry scab; or there may be a scar, the remains of a former ulcer; in more advanced cases there may be perforation of the septum. If there is ulceration it is gerenelly sufficient to rub lightly on the ulcer with cotton-wool wound round a probe and then smear on a slide. If acid-fast organisms are not found, or if no ulcer is present the septum must be scraped with a scalpel till blood appears. Some writers state that *B. lepræ* are found in the nasal septum when bacteriological examination of the body is otherwise negative, or even when there are no other signs of leprosy in the body. The author has seldom found *B. lepræ* in the nose without, after careful search, being able to find them in the skin of the face or ears. Wade and Lara (1927) state 'that of 103 nerve cases, apparently for the most part early, and without bacteriologically positive skin lesions, 3·8 per cent. (4 cases) gave positive nasal smears'.

Another important means of diagnosis in otherwise negative cases is by examination of material taken by puncture from the lymph nodes.

Potassium iodide (Muir, 1928[1]) is an important aid to diagnosis because of its reaction-producing power in hidden foci in skin, nerves, lymph nodes and other organs. In doubtful cases give 20 grains in a large quantity of water twice a week, rising to 240 grains (if a reaction is not provoked by a smaller dose), the dose being doubled on each occasion. In positive cases swelling and pain occur in hidden foci, and in neural leprosy the affected nerve-trunks become thickened and tender, and trophic blisters may appear; if reaction is marked there will be a rise of temperature. Thereafter it is often possible to confirm the diagnosis by bacteriological examination of the reacting part or by testing for changes in the sensory functions. When reactions occur the patient should be examined for an iodide rash and the signs of the lighting up of pyogenic foci in the gums or other parts of the body.

Serological Examination.

Reference has already been made under the description of the organism to serological tests. These have been found to fail in specificity in distinguishing the organism in attempted *in vitro* cultures. Although complement fixation and precipitation tests have been found to be positive in lepers who have reached the B^2 and B^3 stages, using various acid-fast cultures as antigens, these antigens have given negative results not only with non-lepers but also with A_1 and B^1 cases; they are, therefore, of little or no practical diagnostic value, as the diagnosis can be established upon much more sure clinical and bacteriological grounds before any serological test becomes positive.

DIFFERENTIAL DIAGNOSIS.

(1) *From tuberculosis.* There are three situations in which doubt may arise whether a lesion is leprous or tuberculous, viz. in the lungs, in the skin, and in the lymph nodes. In the lungs, leprosy occasionally occurs, pus containing large quantities of acid-fast organisms being coughed up. A patient with such signs is as a rule not so weakened by the condition as he would be if a similar condition were caused by tuberculosis; moreover, in pulmonary leprosy other signs of the disease are invariably present. A much commoner condition is pulmonary tuberculosis in leprosy, and occasionally the lungs are affected by the two diseases simultaneously. In cases of doubt recourse must be had to inoculation of experimental animals. In lupus and tuberculoids of the skin resembling similar leprous lesions, a diagnosis is generally possible upon clinical grounds, but in some cases it is necessary to inoculate guinea-pigs with pieces of the affected tissue. Reference has been made above to the chief features distinguishing the two diseases in the lymph nodes, but here also in cases of doubt the only reliable test is to inject gland tissue into experimental animals. It must be remembered, however, that, as in the lungs, the presence of tuberculosis does not exclude the co-existence of leprosy.

In none of these lesions should the presence of giant cells be taken as pathognomonic of tuberculosis, for as has been shown above, giant cells are found in certain types of leprosy.

(2) *Syphilis.* The relationship of syphilis and leprosy has always been an obscure one. Some writers have classified *syphilitic leprosy* along with nodular, maculo-anæsthetic and mixed as one of four types. There is a strong popular belief that the two diseases have a common origin, and this belief is not without a substantial basis. Very frequently the first signs of leprosy appear shortly after the primary or secondary manifestations of syphilis, and in others, syphilis is accompanied by a marked exacerbation of leprous symptoms. Moreover, many of the lesions of the former disease have a close clinical resemblance to those of the latter.

The problem is rendered still more difficult by the fact that in many cases of all the types of leprosy, both mild and severe, the Wassermann reaction and other serological tests indicating syphilis are found to be positive, although there are no clinical lesions present characteristic of syphilis ; and hence the supposition that leprosy *per se* gives a positive Wassermann.

In the last few years a considerable amount of investigation into this question has been made (Pineda and Roxas-Pineda, 1926 ; Lloyd, Muir and Mitra, 1927) and the evidence goes to prove that :

(*a*) Syphilis and leprosy are ætiologically distinct.

(*b*) Syphilis predisposes strongly to leprosy and therefore latent incubating, or previously unnoticed leprous lesions, are exacerbated by accompanying or superadded syphilitic infection.

(*c*) As a natural corollary, syphilitic infections, giving positive Wassermann reactions, tend to be more common in a leprous than in a general population.

(*d*) The Wassermann reaction is not positive in leprosy when not complicated by syphilis, if a suitable antigen, such as Kolmer's, is used, except for a few weak, false positives. The Kahn precipitation test has been found equally and possibly more reliable in avoiding such false positives.

(*e*) There is reason to believe, though it is difficult to prove absolutely, that in a certain small proportion of B^3 cases the leprous infection prevents the eradication of accompanying syphilitic infection, the serological test becoming negative only as the leprous lesions clear up. Many such cases, however, may correspond to those uncomplicated cases of syphilis, in which the Wassermann does not become negative even under repeated courses of treatment.

The pure syphilitic lesion is not as a rule difficult to distinguish from leprosy, on account of the absence of *B. lepræ*, anæsthesia, nerve thickening, &c.

(3) *Other Diseases.* Leishmaniasis, yaws, tinea, seborrhœa, psoriasis and syringomyelia are among other diseases that are frequently mistaken for leprosy.

FURTHER CONSIDERATIONS.

It is not sufficient to make a diagnosis of leprosy ; for the sake of treatment, prognosis, and prophylaxis, it is necessary to diagnose the type and the phase. Also, before any special remedial measures are attempted it is necessary to seek out and counteract the predisposing causes which are lowering the general resistance of the patient.

Treatment.

As may be gathered from the previous sections of this chapter the chief emphasis in the treatment of leprosy must be laid on the improvement of the general health of the patient, and no line of treatment which neglects this factor is likely to give permanently good results. Syphilis, which is present in India and other places in a proportion varying from 25 to 50 per cent. of cases must be examined for and treated when present. Other predisposing diseases must be sought out and remedied when found. The diet must be carefully enquired into and regulated according to the ordinary rules of dietetics, remembering that leprosy is a disease of over-eating as well as one of starvation. As in tuberculosis, exercise is very important; leprosy is a disease of the lymphatic system and flourishes best in patients with a stagnant lymph-stream and soft, flabby muscles. The hardening up of the muscles is often sufficient of itself to cause a gradual clearing up of the disease. Suitable climatic conditions, general hygienic surroundings and a healthy, cheerful, hopeful and purposeful mental attitude are essentials for successful treatment.

The special treatment of leprosy consists in the application of drugs which break down the defensive mechanism of the leprosy organism and at the same time increase the immunity of the patient. The former of these objects is secured by the injection of such drugs as chaulmoogra (or hydnocarpus) oil and its preparations, and by the administration of potassium iodide orally. It is also promoted by any drugs which cause either local or general polymorphonuclear leucocytosis. The external application of local counter-irritants, for example by painting lesions with trichloracetic acid, which produces leucocytosis in the granuloma, has a beneficial effect. The injection of such irritants as turpentine or the induction of protein shock by the injection intramuscularly or intravenously of milk, bacillary suspensions, and other forms of protein have the same effect. This accounts largely for the innumerable forms of treatment that have been recommended in recent years.

Attempts at the production of immunity have been made by the injection of suspensions of ground-up leprous nodules and of supposed cultures of the leprosy organism, such as Deycke's nastin (Deycke and Reschad, 1907) and Rost's leprolin. Similar endeavours have been made to produce group-specific immunity by the injection of other acid-fast cultures, such as tuberculin, used by many workers, and autolysed tubercle bacilli as proposed by Row (1926). Immunity can, however, be induced (in the opinion of the author, much more effectively and conveniently) by the internal autovaccination caused by the breaking up of the leproma which is produced by the injection of hydnocarpus oil and the oral administration of iodide.

An important point which must be remembered is that in the earlier stages, and especially in B^2 cases, attempts to press this treatment excessively are apt to result in depressing the general resistance of the patient, and thus to lead not to amelioration of the disease but to its aggravation. In B^3 cases this danger, though it exists, is not so great, as patients, because of the higher degree of immunity induced, may continue to improve rapidly under vigorous treatment even when the general resistance is lowered. In all cases, however, there is the danger of the patient reaching an allergic condition with continuous fever, which is one of grave danger and not infrequently ends fatally. In the earliest cases of the A_1 and B^1 types treatment may be pressed, provided the patient's general condition is favourable, seeing that reactions do not occur to an extent likely to depress his vitality.

For the details of treatment as also for the treatment of such complications as leprosy of the eye, nerve reactions, &c., reference must be made to other works, such as the publications of the British Empire Leprosy Relief Association, Indian Branch (Muir, 1928[1]).

Prevention.

GENERAL MEASURES.

As has been mentioned above, leprosy is spread by contact with infectious (chiefly B^2 and B^3 types) lepers, the longer and the closer the contact the greater being the danger of transmission. This being so an infectious patient should have a separate room into which no one but himself enters, except those in attendance on him. His linen, eating utensils, furniture and other things used or touched by him should not be touched by others without due precautions. It is specially important that children should not come in contact with infectious lepers. New-born infants should be separated from their mothers at birth. Those whose occupation entails touching lepers should do so with care ; they should discriminate between different cases regarding the degree of infectiousness, should realize the danger of drop infection while standing near B^3 cases, and should take precautions in dressing ulcerating nodules or in touching or using furniture, utensils, &c., that have been in contact with such patients. Rubber gloves should be worn or the hands should be washed carefully after all possible contacts. At the same time the most important preventive measures are perhaps those which ensure a high measure of physical health.

Children and others who show early signs of leprosy should not be allowed to live in contact with highly infectious cases, as there is good reason to believe that, as in tuberculosis, repeated massive reinfections of those who have only a slight degree of leprosy are apt to lead to a graver type of disease.

Marriage should be forbidden, especially in women, even in the earlier types, as child-bearing frequently leads to a rapid development of the disease. In patients recovering from leprosy, marriage or sexual connection should be interdicted for at least three or four years after all signs of leprosy have disappeared.

PUBLIC HEALTH MEASURES.

The proof of the contagiousness of leprosy has led to the chief emphasis being laid on isolation. Were complete isolation of all persons suffering from the disease possible, doubtless it would disappear from the face of the earth within a generation. But this would demand changes which cannot be expected, not only in national and international laws but also in human nature, and like tuberculosis and other world-wide plagues, leprosy must tarry till human nature and human habits have gradually evolved.

In certain limited and well circumscribed and isolated areas, such as islands, it may be possible to secure good results by complete isolation of infectious cases if there be an enlightened and autocratic government, but where such conditions are absent, as in Africa, India and China, the most hopeful line of procedure is by using as little compulsion as possible and by attracting patients for treatment to leprosy clinics. Whatever opinion may be held with regard to the effectiveness of treatment, there remains now no doubt that we have available remedies which, when properly administered, are sufficiently beneficial to attract large numbers of patients, many of whom continue to attend for months or years because they consider the benefit they receive worth while. Such leprosy clinics (Muir, 1927[2]) may be used as centres of propaganda and education for instructing patients regarding the contagiousness of leprosy and what means should be adopted to avoid the spread of the disease.

Rat Leprosy.

Rat leprosy is discussed here because of its striking resemblance to human leprosy, and because apparently it is capable of producing disease in man. The first account of the causal organism, *B. lepræ murium* (*Mycobacterium lepræ murium*) often called the rat-leprosy bacillus, was published by Stefansky in 1903. Dean (1905) also discovered the organism independently and confirmed Stefansky's work.

B. lep. mur. is strongly acid-fast when stained by Ziehl-Neelsen's method. In size, staining properties and morphology it closely resembles *B. lepræ*, but is a little longer and thinner as a rule. The rods are found intracellularly, but they do not lie parallel to one another to the same extent that they do in the human disease.

Rat leprosy is confined almost exclusively to rats and is found in nature in drain or ship rats (*Mus decumanus* or *norvegicus*), probably because of their dirty and ferocious habits and their cannibalism, which tend to spread infection. White albino rats which are bred from *M. decumanus* are very susceptible, almost 100 per cent. of those inoculated subcutaneously or intra-abdominally developing the disease.

Attempts at *in vitro* culture of this organism do not appear to have been any more successful than those with that of human leprosy. Hollman (1912) reported that he had grown an acid-fast bacillus from the heart-blood of an infected rat on a medium similar to that used by Clegg, viz. in symbiosis with cholera vibrios and amœbæ, and that with this culture he produced lesions in white rats. But this result has not been confirmed. The same difficulty in confirming or refuting the specificity of such cultures does not exist as in the case of supposed *B. lepræ* cultures ; as failure to transmit active, progressive and fatal disease from rat to rat through successive generations (which can easily be accomplished with fresh infected tissue from rats suffering from rat leprosy) may be taken as proof of non-specificity.

Zinsser and Carey (1912) considered that they had obtained an increase of bacilli intracellularly in tissue cultures, but failed to grow the organism extracellularly. Other attempts by Dean, by Marchoux and Sorel (1912) and by Bayon were not successful.

Sugai reported that Japanese dancing mice are susceptible to rat leprosy and Duval confirmed these results.

Two types of rat leprosy were described by Stefansky, one affecting the skin and muscles and the other only the lymph nodes; but Dean declared that there was no marked line of demarcation between the two types, with which opinion the author's experience agrees.

Rat leprosy is found all over the world, due doubtless to the travelling instincts of ship rats; Leger (1919) found it in 1 out of 12 young rats and in 15 out of 37 adults in French Guiana; Marchoux (1912) found it in 65 out of 1,296 animals examined. Ishiwara examined 7,001 rats of various species, but found acid-fast bacilli in 2 only, both of them *M. decumanus.* Of 5,700 rats examined in Porto Rico by Ridlong the disease was found in 3. Marchoux and Sorel (1912) found that in rats living in some quarters of Paris the disease was much more common than in those obtained from other parts of the city. It would, therefore, appear that rat leprosy, like human leprosy, is a world infection and that in both diseases high endemicity seems to gather round certain centres of marked infection.

Marchoux (1922) described the case of a patient suffering from a disease in many respects resembling, while in other ways differing from, human leprosy. Granular acid-fast organisms were found in this case which failed to produce disease in rabbits and guinea-pigs, but caused, when inoculated in white rats, a disease corresponding in every respect with rat leprosy. Our proof of the existence of this disease in man, however, depends so far upon the evidence of this single case.

When tissue suspension containing *B. lep. mur.* is inoculated subcutaneously in rats, definite signs of local intracellular growth begin to show in about three weeks' time. Before this the acid-fast organisms almost entirely disappear, but when once there is a sign of growth, multiplication takes place rapidly, at first in the deeper layers of the skin, whence the more superficial layers are invaded, the normal tissues being gradually replaced by a mass of cells, enlarged and full of organisms. Within 12 to 16 months the whole skin above the point of inoculation is one mass of lepra cells, and the disease has spread through a considerable area of surrounding skin. The interstitial tissue of the muscle underlying the skin is also invaded, and the muscle fibres, though not themselves containing the organisms, become atrophied and disappear, due to the surrounding pressure on them. At this stage a section, when stained by the Ziehl-Neelsen method, shows under the low power of the microscope a uniformly red, granular mass. Later, degeneration takes place at the centre, which then stains less deeply, and afterwards the epithelium is destroyed, and an indolent, discharging ulcer is formed. Before this the affected area becomes hard and depilated, the hair follicles being destroyed.

Within a few weeks of inoculation the nearest lymph nodes show organisms, and a little later the prevertebral nodes and the spleen (Muir and Henderson, 1928).

When inoculation is made subcutaneously, the number of organisms injected makes little difference in the course of events, injection of one-hundredth part of the amount first used producing similar lesions almost as rapidly. There is no evidence that there is any change in virulence even after repeated subinoculations.

Sections stained with hæmatoxylin and eosin show large cells with round or oblong, lightly stained nuclei, similar to those seen in human leprosy. In the spleen the disease follows the line of the blood-vessels, the Malpighian bodies being primarily the seat of attack. Later the disease invades the whole organ, causing by pressure destruction of the spleen cells. The spleen is enlarged to many times the normal size and is covered with white or yellowish nodules. These also can be seen on section.

In the liver the spread is along the portal canals; Kupffer's cells act as hosts and multiply, invading the liver cell areas and destroying them by pressure. To the naked eye, the liver presents a mottled appearance, the yellow, diseased areas showing up against the deep red liver tissue.

When inoculation is performed intra-abdominally the invasion of the peritoneum is most marked. The omentum frequently becomes enormously thickened and nodular (Muir, Henderson and Landeman, 1927), and the serous covering of the intra-abdominal organs becomes covered with small nodules composed of lepra cells full of acid-fasts. Interstitial disease of the kidneys has not been found, but nodulation of the serous covering occurs. The lungs are not as a rule markedly affected, but Marchoux described disease in the apices of the lungs in rats which had acquired leprosy through the gastro-intestinal tract.

When tissues containing *B. lep. mur.* are fed to rats, a certain proportion of infections results, the prevertebral nodes showing the presence of acid-fast organisms some months later.

It is possible that experiments with *in vitro* culture and animal inoculation of the organism of this disease may, if successful, point the way to similar success with that of human leprosy.

The pathological differences between human and rat leprosy are as follows:

(*a*) Human leprosy cannot be transmitted to rats, whereas, apparently, the reverse is possible.

(*b*) Potassium iodide fails to produce any reaction in the rat disease however severely the animal may be infected.

(*c*) The stroma of muscle is invaded in the rat, but not in the human disease.

(*d*) While human leprosy is seldom fatal, the rat disease takes a much more acute form as a rule and is almost always fatal within 12 or 18 months if the rat survives for that length of time after inoculation.

(*e*) There is no evidence of any affection of the nerve-trunks or branches in rat leprosy.

The histopathology of the two diseases, while exhibiting some close points of resemblance which justify the classification of *B. lep. mur.* in the same group as *B. lepræ*, shows also distinct points of divergence, which along with the facts mentioned above make it abundantly evident that the two organisms are specifically, if not generically, distinct.

REFERENCES.

Bayon, H., 1915, *Ann. Trop. Med. Parasit.*, **9**, 1.

Brinkerhoff, W. R. & Moore, W. L., 1909, *U.S. Public Health Bull.*, No. 27.

Dean, G., 1903, *Zbl. Bakt.*, Abt. I, Orig., **34**, 222 ; 1905, *J. Hyg.*, Camb., **5**, 99.

Deycke, Pasha & Reschad, Bey, 1907, *Deuts. med. Wschr.*, **33**, 89.

Duval, C. W. & Harris, W. H., 1913, *J. Med. Res.*, **28**, 165.

Fraser, H., 1913, *J. Trop. Med.*, **16**, 164 ; 1914, *Report to Advisory Committee for the Trop. Dis. Res. Fund*, 5.

Fraser, H. & Fletcher, W., 1915, *Ann. Trop. Med. Parasit.*, **9**, 381.

Hansen, G. A., 1874, *Norsk Mag. Laegevidensk.*, 3 R., **4**, Heft 9, 1–88 ; 1880^1, *Nord. med. Ark.*, **12**, No. 3, 1–10 ; 1880^2, *Virchows Archiv.*, **79**, 32 ; 1882^1, *Nord. med. Ark.*, **14**, No. 29, 9–16 ; 1882^2, *Virchows Archiv.*, **90**, 542 ; 1886, *ibid.*, **103**, 388; 1891, *Internat. Beitr. z. wissensch. Med. Festschr. R. Virchow*, **3**, 61.

Hansen, G. A. & Looft, C., 1895, *Leprosy in its clinical and pathological aspects*, trans. by Norman Walker, Bristol and London, pp. 8, 45, 48, 71, 72.

Henderson, J. M., 1928, *Ind. J. Med. Res.*, **16**, 7.

Hollman, H. T., 1912, *Public Health Rep. U.S. Mar. Hosp. Serv.*, **27**, 69.

India Report of Leprosy Commission, 1890–91, Calcutta, 1892.

International Conf. on Leprosy at Strasburg, 1923, *Report of.*

Kedrowski, W., 1914, *Trop. Dis. Bull.*, **4**, 514.

Leboeuf, A., 1912, *Bull. Soc. Path. exotique*, **5**, 667.

Leger, M., 1919, *Bull. Soc. Path. exotique*, **12**, 169.

Lepra, Bibliotheca internationalis, Leipz., 1900.

Lloyd, R. B., Muir, E. & Mitra, G. C., 1927, *Ind. J. Med. Res.*, **14**, 667.

Marchoux, E., 1911, *Bull. Soc. Path. exotique*, **4**, 89 ; 1922, *Bull. Acad. Méd. Paris*, 3 s., **87**, 545 ; 1925, *Bull. Acad. Méd. Paris*, 3 s., **94**, 1091.

Marchoux, E. & Bourret, G., 1909, *Lepra*, **9**, 63.

Marchoux, E. & Sorel, F., 1912, *Ann. Inst. Pasteur*, **26**, 675, 778.

Muir, E., 1927^1, *Ind. J. Med. Res.*, **15**, 1 ; 1927^2, *Ind. Med. Gaz.*, **62**, 440 ; 1928^1, *Leprosy, Diagnosis, Treatment & Prevention*, Delhi (B.E.L.R.A. Ind. Branch) ; 1928^2, *Ind. J. Med. Res.*, **16**, 135.

Muir, E. & Henderson, J. M., 1928, *Ind. J. Med. Res.*, **15**, 807.

Muir, E., Henderson, J. M. & Landeman, E., 1927, *Ind. J. Med. Res.*, **15**, 15.

Neisser, A., 1880, *Jber. schles. Ges. vaterl. Kult.* (1879), **57**, 65 ; 1881, *Virchows Arch.*, **84**, 514, 1 pl. ; 1886, *Microparasites in Disease* (New Sydenham Soc.).

Pineda, E. V. & Roxas-Pineda, E., 1926, *Philipp. J. Sci.*, **30**, 39.

Rabinowitsch, Lydia, 1903, *Zbl. Bakt.*, Abt I, Orig., **33**, 577.

Rodrigues, J. M., 1926, *Philipp. J. Sci.*, **31**, 115.

Rogers, L. & Muir, E., 1925, *Leprosy*, Bristol & London.

Row, R., 1926, *Trans. Soc. Trop. Med. Hyg.*, Lond., **19**, 407.

Solis, F. & Wade, H. W., 1925, *J. Phil. Is. Med. Ass.*, **5**, 365.

Stanziale, R., 1912, *Lepra*, **13**, 87.

Stefansky, W. K., 1903, *Zbl. Bakt.*, Abt. I, Orig., **33**, 481.

Sugai & Mononobe, 1913, *Tokyo Med. Ass.*, **27**, No. 8, cited from *Trop. Dis. Bull.*, **2**, 287.

Taylor, J. & Malone, R. H., 1924, *Ind. J. Med. Res.*, **12**, 127.

Unna, P. G., 1896, *Histopathology of Diseases of the Skin*, trans. Norman Walker, London, pp. 115, &c.

Wade, H. W. & Lara, C. B., 1927, *J. Phil. Is. Med. Ass.*, **7**, 115.

Wade, H. W. & Rodrigues, J. N., 1927, *A Description of Leprosy*, Culion Medical Board, Philippine Health Service.

Wolbach, S. B. & Honeij, J. A., 1914, *J. Med. Res.*, **29**, 367.

Zinsser, H. & Carey, E. G., 1912, *J. Amer. Med. Ass.*, **58**, 692.

CHAPTER VIII. THE NON-PATHOGENIC ACID-FAST BACTERIA.

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Taxonomy.

THE genus *Mycobacterium* as defined by Lehmann and Neumann (1927) comprises thin, slender rods, frequently with typical branching and sometimes forming branched or unbranched threads. The rods stained with hot carbol-fuchsine are resistant to the subsequent action of acid—that is to say they are ' acid-fast ' and behave with regard to staining as the spores of the ordinary fission-fungi. With some species this acid-fastness may be only feebly developed or, indeed, entirely absent, as in the case of several forms present in soil and instrumental in the decomposition of certain aromatic substances (Gray and Thornton, 1928).

Lehmann and Neumann recognize the following species of these bacteria (other than the organisms of tuberculosis and leprosy): *Mycobacterium smegmatis,* the Smegma Bacillus ; *Mycobacterium lacticola* occurring in 'a,' 'b' and 'c' forms and including organisms isolated from butter, &c. ; *Mycobacterium phlei,* the Timothy-grass Bacillus ; *Mycobacterium eos ;* and *Mycobacterium luteum.*

Bergey (1923), on the other hand, divides these organisms into eight species, as follows : *Mycobacterium smegmatis, Mycobacterium butyricum, Mycobacterium berolinensis* (*sic*), *Mycobacterium friburgensis* (*sic*), *Mycobacterium stercusis* (*sic*), *Mycobacterium phlei, Mycobacterium graminis, Mycobacterium alluvialum* (*sic*). Of these, *M. berolinensis* agrees with the description of Rabinowitsch's Butter Bacillus (Rabinowitsch, 1897), (Lehmann and Neumann's 'c' type of *M. lacticola*), *M. stercusis* with Möller's 'Mist Bacillus', and *M. graminis* with Möller's 'Grass Bacillus II' (Möller, 1899) and Lehmann and Neumann's ' a ' form of *M. lacticola.*

Distribution and Significance.

The saprophytic, non-pathogenic or feebly pathogenic members of the genus *Mycobacterium* are very widespread in nature, occurring in human smegma (*M. smegmatis*), in samples of butter, milk and cheese (*M. lacticola,* &c.), in grass, hay, dust, &c. (*M. phlei*) and in dung, &c. (*M. stercusis*). It is stated by Rabinowitsch that 60 per cent. of butter samples in Berlin contain saprophytic acid-fast bacteria. Similar organisms have been isolated by A. Eastwood and F. Griffith (1912) from butter samples in this country. These organisms are only of importance in so far as they may be confused, under certain conditions, with tubercle bacilli, or if they give rise, in the course of experimental inoculation of animals, to lesions which resemble those of true tuberculosis.

Morphology and Cultural Characters.

In general morphology, staining reactions, &c., many of these organisms resemble the tubercle bacillus, but they are in consequence of their proportionately quicker growth on the usual culture media (agar, glycerin-agar, &c.), and their capacity to grow at lower ranges of temperature (25 to 30° C.) readily distinguished. Some of them, however, show considerable points of resemblance to the tubercle bacillus, which may sometimes cause difficulty in differential diagnosis. *M. smegmatis*, for example, originally described by Alvarez and Tavel (1885), and notable as the first acid-fast organism to be discovered after the tubercle bacillus of Koch, is of some practical interest, in that it may sometimes be confused with the latter organism. When present in smegma it appears to be easily decolorized with alcohol (Weber, 1903), but the strains subsequently isolated by Möller (1902), two of which are conserved in the National Collection of Type Cultures (1925) are strongly alcohol- as well as acid-fast, that is to say they are not decolorized by a 3 per cent. solution of acid-alcohol after Ziehl-Neelsen staining. In the case of *M. smegmatis* also, the comparatively rapid growth, and the negative results of guinea-pig inoculations are important points in differential diagnosis.

The saprophytic acid-fast organisms are relatively fast growing and grow well on all ordinary laboratory media. The growth on agar slopes at 30° or 37° C. is abundant, often wrinkled or creamy, ranging in colour from pale cream to yellow, orange or red. Many strains show towards the periphery of the culture a delicate wavy border zone. Broth cultures remain clear, while a yellow or orange pellicle, which mounts a short way up the side of the test-tube, is often present on the surface. Gelatin is not liquefied. Litmus milk is generally acidified but not coagulated, an orange-coloured ring being frequently formed. Many strains give abundant, brightly pigmented growth on Loeffler's solidified blood-serum.

Pathogenicity.

Some of these Saprophytic Mycobacteria appear to be pathogenic to laboratory animals under experimental conditions.

M. phlei, for example, is lethal to guinea-pigs and rabbits by intraperitoneal injection of 3 to 4 c.cm. of broth culture. The resulting lesions resemble those seen in true tuberculosis, caseating nodules containing giant-cells being often present in the internal organs. Many of the organisms isolated from butter, grass, &c., are also feebly pathogenic to such laboratory animals. A small mass of culture inoculated subcutaneously into a guinea-pig, for example, may produce a local abscess, which after a couple of weeks breaks open and heals spontaneously. Solitary nodules may be present in the neighbouring lymphatic glands and internal organs (liver, &c.). The process is not usually fatal and even when massive inoculations are made into the peritoneal cavity the lesions frequently heal. According to Rabinowitsch, animals with such peritoneal injections, if killed some 3 or 4 weeks afterwards, exhibit slightly swollen abdomen and more or less severe peritonitis, the peritoneum being studded with small nodules. Numerous nodules are also found under the abdominal serosa. The mesenteric glands are considerably swollen and are often caseous. Numerous nodules are also present in the spleen, liver, kidneys and lungs.

If, in addition to the pure culture of the organism in question, 4 to 5 c.cm. of butter fat at 37° C. is injected, fatal effects may occur in from 3 to 15 days. Intense fibrinous peritonitis is often present, the exudate containing numerous acid-fast bacilli (Mayer, 1899 ; Aujeszky, 1904).

Pure cultures of Möller's smegma bacillus, on the other hand, appear to be devoid of virulence for laboratory animals (Möller, 1902). Aujeszky (1904), in confirming Möller's results, found that the intraperitoneal inoculation of culture material together with sterile butter was occasionally pathogenic to rabbits. It seems reasonable to conclude, however, that these saprophytic organisms have no significance in relation to human disease.

No proofs have ever been brought forth to show that true tubercle bacilli can be evolved from free-living species of Mycobacteria, although several workers (Kolle, Schlossberger and Pfannenstiel, 1921 ; Sanfelice, 1920 ; Schröder, 1921), claim to have increased the virulence of saprophytic forms by means of guinea-pig passage and to have eventually developed strains in complete biological agreement with *M. tuberculosis* Koch. These results have not, however, been confirmed by subsequent workers, and it is impossible to exclude the probability that the experiments were vitiated by the presence of spontaneous guinea-pig tuberculosis.

REFERENCES.

ALVAREZ & TAVEL, 1885, *Arch. physiol. norm. path.*, 3 s., **6**, 303.

AUJESZKY, A., 1904, *Zbl. Bakt.*, Abt. I, Orig., **36**, 415.

BERGEY, D. H., 1923, *Bergey's Manual of Determinative Bacteriology*, Baltimore (Williams & Wilkins Co.).

EASTWOOD, A. & GRIFFITH, F., 1914, *Rep. Med. Off. Loc. Govt. Bd. for* 1912–13, **42**, 299.

GRAY, P. H. H. & THORNTON, H. G., 1928, *Zbl. Bakt.*, Abt. II, **73**, 74.

KOLLE, W., SCHLOSSBERGER, H. & PFANNENSTIEL, W., 1921, *Deuts. med. Wschr.*, **47**, 437.

LEHMANN, K. B. & NEUMANN, R. O., 1927, *Bakteriologie*, Band II, München (J. F. Lehmann).

MAYER, G., 1899, *Zbl. Bakt.*, Abt. I, Orig., **26**, 321.

MÖLLER, A., 1899, *Zbl. Bakt.*, Abt. I, **25**, 369 ; 1902, *ibid.*, Abt. I, Orig., **31**, 278.

NATIONAL COLLECTION OF TYPE CULTURES, 1925, Catalogue, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. **64**, 2nd ed., revised.

RABINOWITSCH, L., 1897, *Z. Hyg. InfektKr.*, **26**, 90.

SANFELICE, F., 1920, *Ann. Igi.* (*sper.*), **30**, 1.

SCHRÖDER, G., 1921, *Z. Hyg. InfektKr.*, **94**, 493.

WEBER, A., 1903, *Arb GesundhAmt.*, Berl., **19**, 251.

CHAPTER IX. THE BRUCELLA GROUP.

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Introduction.

BY J. T. DUNCAN AND L. E. H. WHITBY.

THE genus *Brucella* includes a number of small coccoid or cocco-bacillary organisms which are non-motile, non-sporing and Gram-negative; which form neither acid nor gas from carbohydrates and tend to produce an alkaline reaction in litmus-milk.

The generic name is derived from that of Sir David Bruce, who in 1887, first described and incriminated *Micrococcus melitensis* as the cause of Malta fever. Other species of the genus are *Brucella abortus* described by Bang in 1897 as the cause of epizootic abortion in cattle, and named by him *Bacillus abortus*, and *Brucella paramelitensis*, a variety of *Brucella melitensis*, reported by Nègre and Raynaud in 1912. Minor variants of all three organisms are common.

Bergey, following the nomenclature of Castellani, has grouped together as the genus *Alkaligenes*, *B. fæcalis alkaligenes*, *B. bronchisepticus*, *Br. abortus*, *Br. melitensis* and *Br. paramelitensis*, taking the first named as the type. There is little justification for this classification save that the power of alkaligenesis is common to all.

The type of the genus, *Br. melitensis*, produces in man a clinical entity characterized by prolonged and irregular fever, and the disease was at one time thought to be confined to the Mediterranean littoral, being particularly rife in Malta. Hence there are in common use the terms Malta fever, Mediterranean fever, Rock fever, Gibraltar fever and Neapolitan fever. Investigation having shown that the infection is widespread throughout the world, it is preferable to abandon the local names, and to refer to the disease by the term 'undulant fever', as suggested by Hughes (1896).

History.

BY W. BULLOCH.

We owe a large part of our knowledge of Malta fever to English bacteriologists. It was in 1887 that David Bruce, working in conjunction with a Maltese, Caruana-Scicluna, first saw the micro-organism which causes the disease. He related the history of five cases, in the first of which he was able to show the existence of numerous minute cocci in paraffin sections of the spleen. In the remaining four he succeeded in cultivating the coccus from the spleen on agar, and he described and figured its characteristic manner of growth. Continuing his observations, Bruce (1888) isolated the coccus in a sixth case, and by means of a culture reproduced the disease in a monkey, from the spleen of which he regained the coccus pure, after the animal had died on the twenty-first day. Rabbits, mice and guinea-pigs yielded, on the whole, negative results. From one rabbit, however, Bruce recovered the coccus 26 days after inoculation. The observations of Bruce were confirmed by Gipps (1891) and by L. Hughes (1892, 1893), who also successfully inoculated monkeys. Bruce and Hughes together had four successful inoculations out of a total of seven monkeys (Bruce, 1893). The micro-organism was named by Bruce (1893), *Micrococcus melitensis*. The further proof of the ætiological significance of the coccus in man was brought by accidental or intentional inoculation in man. D. Semple was accidentally infected in 1897, and A. E. Wright (1897), having inoculated himself with dead cultures, injected 1/100 of an agar culture of a living culture of Malta fever coccus, the result being a sharp attack of the disease (Birt and Lamb, 1899). Many workers have contracted this disease accidentally, and in at least one case (T. Carbone) with a fatal result. In 1897, A. E. Wright and F. Smith demonstrated the existence of specific agglutinins and thereby greatly improved the accuracy of the diagnosis of Malta fever, and it was soon found to have a wide geographical distribution. There still remained, however, a good deal of doubt with regard to the manner of infection. A Commission was appointed in 1904 by the Admiralty, War Office and the Civil Government of Malta to investigate the disease still further, and the direction was placed under the Chairmanship of David Bruce. Between 1905 and 1907, the active workers of the Commission published seven reports which threw a flood of light on the subject. The main results were improvements in the bacteriological technique, the demonstration of the saprophytic existence of *Micrococcus melitensis*, its existence in the urine and, strangest of all, the proof of the existence of specific agglutinins and Malta fever cocci in the milk of goats. The transference of the disease from goats' milk to monkeys was successfully achieved, and a striking confirmation of the new ideas was afforded by the outbreak of Malta fever on board S.S. ' Joshua Nicolson ', which was carrying goats from Malta to Antwerp, en route for the United States. Of 10 of the crew who drank the goats' milk, 8 developed Malta fever.

The most recent development in the Malta fever question has been the attempt to link it up with the organism of epizootic abortion discovered by B. Bang (1897). This has been chiefly the work of Alice C. Evans (1918) and Meyer and Shaw (1920). Serological races of the coccus of Malta fever have been described as ' pseudomelitensis ' Sergent, Gillot and Lemaire (1908), or ' paramelitensis ' Nègre and Raynaud (1912).

Morphology.

BY J. T. DUNCAN AND L. E. H. WHITBY.

The type species, *Br. melitensis*, was described originally by Bruce (1887), its discoverer, as a small micrococcus occurring singly, in pairs or in short chains, dancing about. A bacillary form was, however, recognized by Durham (1898) and by Gordon (1899), who also quotes Horrocks's description of the organism as 'in reality a small bacillus'. At the present day, although the original name *Micrococcus melitensis* is still widely used, all but a few bacteriologists regard the organism as a small bacillus. In recently isolated strains of *Br. melitensis*, especially those of Mediterranean origin, the coccoid form of the organism predominates in the cultures, but, with the progressive development of the saprophytic state in artificial cultivation, the short bacillary form tends to become pronounced, and, for this reason, may be regarded as the more perfect form of the organism.

In shape the brucellas are coccoid or bacillary cells with rounded or oval ends, appearing in smear preparations as isolated cells or in pairs; but chains of 5 to 8 or even 14 individuals may be found in smears from cultures in liquid media. In structure they show refractile polar condensations, which may be seen in the larger forms when viewed in living wet preparations, or in fixed smears lightly stained with carbol fuchsine, when the poles of the organism appear more deeply stained than the centre. This appearance, which is more marked in *Br. abortus* than in the other types, led Nowak (1908) to remark on the resemblance of this rganism to the pasteurellas.

The size of *Br. melitensis* and *Br. paramelitensis* varies between 0·4μ in thickness by 0·6μ in length in the coccal forms, and 0·5μ by about 1·5μ in the bacillary forms. *Br. abortus* in the coccal form is similar in size to the other two species, but its longest bacillary forms, developed on a rich medium, may attain a length of 2·5μ or 3·0μ: Bang (1897) and Mohler and Traum (1911) give the length of this species as up to 3·0μ, but, on the other hand, Fabyan (1912) states that he has rarely found it to attain to a length of 1·5μ.

All types of the genus are polymorphic, and stained preparations from a single culture may show simultaneously coccal and bacillary forms of various sizes occurring as isolated cells, in pairs or in chains. Except that chain grouping occurs in liquid media, the nature of the culture medium seems to have but little influence on morphology, which apparently depends chiefly upon the type of organism, its intrinsic tendency to variation, and, perhaps, in a lesser degree on the age of the strain. The morphology of the organisms in smears from exudates is similar to that of the cultivated strains, and may show polymorphism. Involution forms in cultures of the brucellas are of unusual occurrence, unless the suggestion of Eyre (1925), that the bacillary forms of *Br. melitensis* are involutionary, can be accepted. Rarely, however, in well grown cultures apparent involution bodies may be seen. These usually take the shape of large cocci, about 1·0μ in diameter, sometimes occurring in short chains and often showing a light equatorial band suggesting the appearance of the neisseria.

Although *Br. abortus* is larger and more rod-like in shape than the other types of the genus, it is not possible, except perhaps with the largest bacillary forms of *Br. abortus*, to identify the types on morphological evidence alone.

Staining.

All types of the genus stain well with the ordinary basic aniline dyes. They are Gram-negative and not acid-fast. One of the most suitable stains is the Ziehl-Neelsen carbolic fuchsine diluted to about 20 per cent. strength with distilled water. In the case of *Br. abortus*, Fabyan (1912) has pointed out that carbol fuchsine staining tends to accentuate the apparent thickness of the organism and to make it more coccus-like, while methyl-violet accentuates the apparent length and emphasizes the bacillary form; possibly conflicting descriptions of the morphology of the organisms may have been due partly to lack of appreciation of this factor.

Intense staining with carbol fuchsine stains the larger bacillary forms uniformly, giving them a solid rod-like appearance. If now the preparation be treated rapidly with alcohol the central zone of the organisms becomes partly decolorized, while the poles retain the stain, giving them a pasteurella-like appearance. A more prolonged application of alcohol may completely decolorize the centre of the organism and give rise to the false impression of a pair of cocci. The paired cocci seen in normally stained preparations do not, however, owe their appearance to any staining artefact.

Cultivation.

GENERAL BEHAVIOUR OF THE ORGANISMS IN ARTIFICIAL CULTURE.

Optimum temperature, &c. The optimum temperature for the cultivation is about 37° C., but laboratory strains established in saprophytic growth, will after the culture has been started at 37° C., on a favourable medium, continue to develop slowly at room temperature (16 to 19° C.) for upwards of six weeks. Perceptible growth may also occur up to 42° C., but no growth takes place at 45° C. or as low as 6° C.

Reaction of the medium. Depending in some measure upon the nutrient value of the medium, the age of the strain and the mass of bacteria sown, the brucellas will grow in media having an extreme range of reaction from pH 6·2 to pH 8·8. The normal range for laboratory cultures on ordinary media is from about pH 6·6 to pH 7·8 or pH 8·0, and the optimum reaction is about pH 7·4. In all cases growth is followed by an alkaline change until the reaction reaches about pH 8·0 or a little over. This change in certain media, especially in the case of old strains, as shown by Huddleson and Winter (1927), may be associated with the formation of crystals of ammonio-magnesium-phosphate. If a good agar medium, set to a reaction of pH 7·2 or pH 7·4, and tinted with phenol-red or brom-thymol-blue as an indicator, be sown with one of these organisms, it will be found that the earliest change in reaction is a slight acidity developing in the vicinity of the first colonies and spreading gradually into the depth of the medium. With a heavy inoculation yielding a rich growth, this acid change develops rapidly, but, as alkaline bodies are formed the reaction swings back to its starting-point, which is reached in about 72 hours; after this the alkaline change progresses until growth ceases.

The brucellas as a whole are more tolerant of alkali than of acid, but most strains of *Br. abortus* seem to be slightly more acid-tolerant than *Br. melitensis*, especially if the sowing be heavy, for with *Br. abortus*, more than with *Br. melitensis*, co-operation between the individual organisms apparently plays some part in establishing the growth of the culture.

Humidity of the medium. An excess of moisture may lead to an earlier growth of the organism, but not to luxuriance of growth; the culture will spread within the confines of the moist surface, but usually remains as a relatively thin layer. On a dry medium the initial growth is slower and is limited to the area of inoculation, but it may continue to increase in thickness for several weeks, presenting at the end of that time an elevated plaque, often of a brownish colour, with steep, sharply defined margins.

Relation to oxygen supply. The brucellas, except certain freshly isolated strains of *Br. abortus* from bovine sources, grow most freely at the normal oxygen tension of air, and do not usually develop under anaerobic conditions. There exists, however, some divergence of opinion regarding the atmospheric needs of the bovine strains referred to. It was formerly thought that they were micro-aerophil because of their failure to develop in normal air, but this failure seems to be due not to a relative excess of oxygen but to the need of a higher tension of carbon dioxide. The early successful attempts of Bang, Stribold, Nowak and Fabyan to cultivate *Br. abortus* under conditions of reduced oxygen tension involved the use of closed vessels in which, as explained below, the carbon dioxide tension would gradually increase; Holth obtained equally successful results by using an increased oxygen tension, and Preisz (1903) cultivated the organism from tissues on agar surfaces in sealed tubes to which oxygen had been added previously.

It is generally accepted that the brucellas will not grow under anaerobic conditions, but it seems possible that some strains may be facultative in this respect, for Horrocks (1905^1) mentions that the anaerobic growth of *Br. melitensis* is feebler than the aerobic, and T. Smith (1924) has grown *Br. abortus* in atmospheres of 100 per cent. carbon dioxide, 10 per cent. carbon dioxide + 90 per cent. nitrogen, and 10 per cent. carbon dioxide + 90 per cent. hydrogen. He admits, however, that traces of free oxygen may have been present in all these gases.

Relation to carbon dioxide supply. Normal strains of *Br. abortus* of bovine origin cannot be grown in primary culture or early subculture at the ordinary carbon dioxide tension of the air, and to ensure growth it is necessary to raise the proportion of carbon dioxide to 5 or 10 per cent. by volume. The carbon dioxide requirement of individual strains varies considerably, but diminishes as the strain is propagated on artificial media, until after about 16 to 20 subcultures it will grow readily at the normal carbon dioxide tension of the air. It remains thereafter a free-growing strain, and the original character cannot be restored even by prolonged sojourn in an artificially infected animal.

The necessary increase of carbon dioxide may be attained in various ways, the simplest being to add to a vessel, such as a Bulloch's jar, in which the cultures have been placed for incubation, a sufficient amount of pure carbon dioxide to give the optimum concentration. Nowak (1908) grew *Br. abortus* on the surface of solid media by incubating the cultures in a closed vessel with cultures of *B. subtilis*, which give off an appreciable amount of carbon dioxide in their growth. The mass of *B. subtilis* culture must be regulated, for a very small growth may not yield sufficient carbon dioxide, while an excessive growth may arrest the growth of *Br. abortus* by exhausting the oxygen. Nowak found the optimum to be 1 sq. cm. of *B. subtilis* growth to every 15 c.cm. of air space. Any micro-organism yielding carbon dioxide, even a saprophytic strain of *Br. abortus*, may be used instead of *B. subtilis*.

That the culture itself may be able to provide the necessary increase in carbon dioxide is suggested by the conditions under which the organism was cultivated by Bang, Preisz and others. Bang (1897) isolated *Br. abortus* originally as a shake culture in a rich semi-solid medium containing agar, gelatin and fresh uncoagulated serum. In this medium, after 2 to 4 days' incubation at 37° C. in an unsealed tube, growth, in primary culture, appears as a zone of small colonies commencing about 5 mm. below the surface of the medium and extending to a depth of 15 to 20 mm. The organisms at this stage could not be grown as surface cultures in unsealed tubes. Richness of the medium and the necessity for increasing the carbon dioxide seem to some extent to be complementary, but even with the richest media some increase of carbon dioxide is necessary. It may be possible to explain the growth in Bang's medium by assuming that a sufficient amount of carbon dioxide is given off by the dead and disintegrating organisms in the inoculum to enable some of the survivors to commence growth. These growing organisms in turn evolve carbon dioxide, which aids the growth of other and more exacting organisms, until finally a suitable environment is provided for the full development of the culture. Near the surface of the medium the loss of carbon dioxide by diffusion prevents growth, and below the zone of growth conditions may not be sufficiently aerobic.

Preisz (1903) cultivated *Br. abortus*, from its isolation, as a surface growth on agar and other media by merely sealing the culture-tube, which prevents loss of the carbon dioxide formed. This work was followed up by Fabyan and greatly elaborated by Theobald Smith. Success in culture of recent strains in sealed tubes depends to some extent on the richness of the medium and on the mass of bacteria sown; a relatively light sowing may yield no growth when a heavier one succeeds. When growth follows a relatively light sowing the colonies appear in a succession of crops, as the accumulating carbon dioxide reaches concentrations more and more favourable until the optimum is reached, as found by analysis.

In what manner carbon dioxide aids the growth of *Br. abortus* is not understood, but it seems possible that it may be as a source of carbon. In closed vessels it definitely regulates the reaction of the medium by combining with alkaline bodies resulting from the growth of the organism, and thus delays or prevents the alkaline change in the medium which eventually arrests growth and may even lead to the death of the culture.

There are records by various writers of the cultivation of *Br. abortus* directly from cows' milk and the uterine discharges of aborting heifers at the normal carbon dioxide tension of air, but these instances are exceptional. In the case of *Br. abortus* of porcine origin, however, it is the rule for the organism to grow freely in normal air from the start, and this applies also to the type of *Br. abortus* infecting both cattle and man in Rhodesia.

Neither *Br. melitensis* nor *Br. paramelitensis* exhibits this peculiar need of carbon dioxide, and they, in common with those strains of *Br. abortus* which have acquired the capacity to grow in normal air, develop more freely in plugged but unsealed tubes under normal air conditions than when carbon dioxide is added or the tubes are sealed. If such strains be cultivated in sealed tubes until growth ceases, a renewal of growth usually occurs after the seal has been broken. All types are unfavourably affected by high concentrations of carbon dioxide, and in the presence of 80 to 90 per cent. of the gas growth is retarded, and in the case of some strains definitely inhibited.

SUITABLE CULTURE MEDIA.

All types of the genus will grow readily, although somewhat slowly, on simple media such as nutrient agar. A very rich yield of growth may be obtained on vitamin or blood-digest media, but perhaps the most suitable medium for general work, and for conserving cultures is glucose agar, prepared by adding 2 per cent. glucose to a simple meat extract agar medium set to a reaction of pH 7·4. To obtain a high yield of growth in a short time, one of the most useful media is peptic-digest-blood-agar (see Vol. IX), on which, even in the case of freshly isolated strains, a rich growth can be obtained within 24 hours. Another good medium for obtaining an early and rich yield of growth is glucose-nutrose-agar (glucose-agar with 1 per cent. of nutrose).

All of the brucellas grow freely in the presence of bile, and bile media are useful for the isolation and enrichment of the organisms in primary culture; Stafseth's (1920) liver infusion medium is excellent for this purpose.

Gentian violet in a concentration of one part of a saturated watery solution of the dye to 10,000 parts of the medium is strongly inhibitory to many of the Gram-positive cocci, and it is used sometimes for the enrichment of primary cultures of *Br. melitensis* and *Br. paramelitensis* Huddleson and Abell (1928) have shown, however, that the varieties of brucella exhibit differences in the degree of their resistance to this dye; *Br. melitensis* growing freely in the presence of a concentration as strong as 1 : 50,000, while *Br. abortus* bovine strains may be inhibited by this concentration and grow rather poorly at 1 : 100,000, and the porcine strains are still less resistant and may not grow in the presence of a 1 : 100,000 concentration.

THE CHARACTERS OF THE GROWTH OF *BR. MELITENSIS* AND *BR. PARAMELITENSIS* ON PARTICULAR MEDIA.

Simple nutrient agar. On simple agar the colonies appear in 2 to 4 days at 37° C. as minute translucent points which, from the first, show considerable irregularity in size. They are sharply circumscribed and elevated, develop slowly, and, as they enlarge become opalescent, so that the fully developed colony has been aptly compared to a droplet of mucus. Isolated colonies after 10 to 14 days' growth may attain a diameter of 1·0 to 2·0 mm. As the culture ages the colonies become thicker and more opaque, presenting a buffy appearance, or even a brown coloration in cultures a month to six weeks old. When the sowing has been heavy, growth may appear in 24 up to 48 hours, as a thin filmy veil made up of colonies varying greatly in size, and usually presenting a continuous margin. As the culture develops the colonies near the margin coalesce and form a thick continuous and somewhat raised edge showing no tendency to spread, while growth in the centre remains relatively thin and the colonies may in places remain discrete. A stab culture in simple agar, incubated at 37° C., shows a small surface growth with an extension along the needle track for about 1·5 cm. after 48 hours. Within 7 days a light growth may be seen extending along the greater part of the needle track, but the denser growth does not extend beyond about 1·5 cm. from the surface.

Glucose agar. On a glucose agar slope, heavily sown, growth appears in about 24 hours, or it may be delayed to 48 hours. It appears as a continuous film, whitish at first and presenting a well-defined raised margin if the surface of the medium is not very moist. After a few days' incubation at 37° C. the culture may be maintained at room temperature (16 to 19° C.), when growth will progress slowly for 6 weeks or longer, developing into a thick raised plaque with fairly steep rounded edges and a shiny surface of a buffy or even brownish colour. Secondary colonies may appear in the later stages of growth.

Specially rich media. On rich media such as Fildes's peptic-digest-blood-agar or digest-liver-agar, growth occurs early and is very luxuriant, appearing as isolated colonies of the type described, or as a well developed whitish film, in 16 to 24 hours. The film develops rapidly into a thick greasy-looking greyish growth. Cultures on these rich media, especially in the case of old strains, when free access of oxygen is allowed, develop a marked alkaline reaction very early, and this change is sometimes associated with the formation of crystals of ammonio-magnesium-phosphate in the growth and just under the surface of the medium, visible with a hand lens or even to the naked eye in cultures 48 to 72 hours old. The conditions associated with the early formation of these crystals retard or arrest growth, and may lead to the death of the culture. For this reason, strains propagated on very rich media should be subcultured frequently, about twice monthly.

Gelatin. On a gelatin slope at 20° C. the cultures develop very slowly. The time of appearance of the earliest visible growth depends upon the density of the sowing, but is usually about 2 or 3 weeks. The gelatin is not liquefied, and the general appearance of the culture is similar to, but much less luxuriant than, that on agar. Ageing cultures may develop a brownish pigmentation. In gelatin, as in agar, a stab culture shows the greatest amount of growth at the surface and in the upper part of the needle track, with a light growth in the deeper parts. The appearance is very similar to that of an agar stab culture.

Glucose gelatin. A shake culture in 2 per cent. glucose gelatin offers one of the best means of conserving stock cultures. The inoculated medium should be incubated at 37° C. for 2 or 3 days, when a light diffuse growth, with perhaps some deposit, occurs. The tubes may then be removed from the incubator and the gelatin allowed to set. If the cultures are now maintained at room temperature (16 to 19° C.) a further growth becomes apparent after a week or 10 days, forming a zone of minute colonies extending from the surface, or about 1·0 mm. below it, to a depth of about 5 to 8 mm., which, after several weeks, presents a dense cloudiness. Below the zone of dense growth are isolated colonies diminishing in number and size as the deeper parts are reached.

Potato. Neutral or slightly alkaline potato sown from an agar culture of any type of the genus yields, in 24 to 48 hours at 37° C., a visible growth of a smooth white glistening character, which acquires a light cream or honey yellow colour by the end of a week. Well-established growths allowed to develop slowly at room temperature, form, after a few weeks, a thick raised plaque with steep rounded margins, and varying in tint from a deep honey colour to a chocolate brown. This pigmentation is very striking, and it has, in the case of the *abortus* type; been compared by McFadyean and Stockman (1909) to the appearance of a culture of *B. mallei* in a similar medium.

After several weeks' growth secondary colonies may appear on the surface of the growth as minute papillæ.

On alkaline potato (pH 8·5) differences in the growth characters of *Br. abortus* and the other brucellas may sometimes be found in week-old cultures, if the sowing has been light, *Br. melitensis* and *Br. paramelitensis* usually yield a relatively poor growth of a greyish or buffy colour with patches of deeper pigmentation, varying from chocolate colour to black, while *Br. abortus* yields a much more luxuriant growth of a uniform creamy yellow colour (Meyer and Shaw, 1920). Unfortunately these differences are not sufficiently constant to be of use in distinguishing the types.

Nutrient broth. In a simple broth medium at 37° C. growth appears as a light cloudiness in about 24 hours, followed during the next few days by an increasing turbidity, and later by a deposit, or bottom growth, at first light and powdery but becoming tenacious and ropy. After about the second week the medium begins to clear gradually as the growth sinks to the bottom. In some strains, however, the turbidity is of a more permanent character. A surface ring or a light pellicle is rarely to be found. The nutrient value of the broth is greatly increased by the addition of 1 to 2 per cent. of glucose.

Milk. The organisms grow freely in milk, but the growth is not so profuse as that in broth. In skimmed milk, tinted with an indicator such as phenol red, an alkaline change is apparent after about 7 to 10 days' growth at 37° C., the pH rising from say 6·6 to 7·6. The degree of alkalinity may increase slightly up to the end of the third week. There is no preliminary acid change and no clotting or curdling of the milk. If whole milk be used instead of skimmed milk, the thick cream layer, by excluding free access of oxygen, may prevent the alkaline change. There is no apparent difference in the degree of alkalinity caused by the different brucellas.

MAINTENANCE OF CULTURES.

Strains maintained on digest-liver media must be subcultured frequently, about twice monthly. If a simple glucose-agar culture, after about 2 days' incubation at 37° C., be sealed with a well-fitting rubber cap or by paraffin wax, it may be conserved in the living state for upwards of two years. Glucose-gelatin shake cultures incubated for 2 days at 37° C., and when sealed or capped and stored at room temperature will remain viable for at least 18 months.

Biochemical Reactions.

ACTION ON PROTEINS.

Milk. A slow alkaline change, without clotting, in separated milk is caused by all types of the genus. There is no liquefaction of gelatin or of blood-serum. Indole is not formed.

The formation of hydrogen sulphide from proteins or amino bodies containing sulphur is one of the most important biochemical reactions.

Its value as a means of distinguishing *Br. abortus* from the other types has been pointed out by Huddleson, Hasley and Torrey (1927), who recommend for the test the use of Stafseth's liver-infusion-agar medium, which is relatively rich in organic sulphur. The medium should be set to an initial reaction of pH 6·6 and, after sowing, a strip of lead acetate paper should be introduced into the tube. After 24 to 48 hours' incubation at 37° C. a distinct blackening of the lead acetate paper occurs in the tubes sown with *Br. abortus*, while those exposed to the cultures of *Br. melitensis* and *Br. paramelitensis*, as a rule, show no change, or at most a slight blackening of the edges of the paper. Occasionally an old strain of *Br. melitensis* may cause a considerable blackening of the paper. It is recommended that the tubes should not be sealed, but access of air permitted, as, it is explained, the failure of *Br. melitensis* to form free hydrogen sulphide is due to an early alkaline change in the medium caused by the rapid growth of this species ; any hydrogen sulphide formed unites with the alkaline bodies in the medium to form non-volatile sulphides. In the case of *Br. abortus*, however, the alkaline change is delayed and free hydrogen sulphide is evolved for 24 to 48 hours. On the other hand, if the tubes are sealed the retained carbon dioxide given off by the growing culture arrests the alkaline change, and free hydrogen sulphide might be given off by cultures of *Br. melitensis*. Duncan (1928) confirms the value of this test as an aid in differentiating *Br. abortus* from the other brucellas, when recently isolated strains of the organisms are employed. He found, however, that the evolution of hydrogen sulphide by cultures of *Br. abortus* ceased while the medium was still acid, and that normal recent strains of *Br. melitensis* or *Br. paramelitensis* did not tend to form hydrogen sulphide in appreciable amount whether the tubes were left unsealed or were capped, even although the medium remained slightly acid for several days. The use of capped tubes also enables the test to be applied to those strains of bovine *Br. abortus* which cannot as yet be grown in unsealed tubes in ordinary air.

ACTION ON CARBOHYDRATES, &C.

The brucellas are deficient in the power to ferment the common sugars, alcohols, &c. They do not ferment glucose, lævulose, lactose, maltose, galactose, raffinose, saccharose, xylose, mannitol, dulcitol, inositol, dextrin, starch or salicin, but some strains cause, after 4 to 8 days' delay, a slight acid change without gas formation in 1 per cent. arabinose peptone-water medium. The degree of acidity varies with individual strains and many show no action whatever. Fermentation of this sugar is not in any way correlated with the type of brucella.

Although there is no gross fermentation of glucose with acid change, McAlpine and Slanetz (1928) have shown that all the brucella varieties except the bovine strains of *Br. abortus* are able to decompose and utilize the available glucose in the culture medium.

ACTION ON SALTS OF ORGANIC ACIDS.

The brucellas do not appreciably decompose the soluble salts of any of the following organic acids: succinic, glutaric, pimelic, fumaric, maleic, aconitic, glycollic, malic, d-tartaric, l-tartaric, m-tartaric and mucic acids.

Reductions, &c. The brucellas do not reduce nitrates or neutral red.

Viability and Persistence.

The brucellas may survive in a living state, without multiplying, in soils, in water and on cloth fabrics, and the duration of survival seems to be as great in the case of freshly isolated strains as in old saprophytic ones. Horrocks (1905–6) and Gilmour (1906), investigating the viability of *Br. melitensis* in the soil and water of Malta, found it to survive for the following periods in the media specified: Sterilized tap-water, up to 42 days; unsterilized tap-water, 7 days; sterilized sea-water, 25 days; damp sterilized soil, 72 days; dry sterilized manured soil, 69 days; moist sterilized manured soil, 7 days; unsterilized manured soil, 20 days; dry natural road dust, 28 days; dry sterilized sand, 20 days; on dry cloth, 80 days, and on dry sterilized glass slides, 16 days.

Exposure to direct sunlight is lethal in one hour. Practically all strains, if in a state of dispersion in a liquid medium, are quickly destroyed at a temperature of 59° C., but strains are met with which are susceptible to lower temperatures down to 56° C.

The organisms will survive in 0·05 per cent. hydrochloric acid for long periods, but are killed by 0·5 per cent. in less than an hour. Gastric juice exerts a slight inhibitory effect.

Br. melitensis has, in the urine of the patient, a long survival period attributable to the high acidity so commonly present in undulant fever; in normal urine the development of an alkaline reaction kills off the organism in about seven days.

All the brucellas may survive for some months in pieces of animal tissues preserved from putrefaction. Mohler and Traum (1911) mention the recovery of *Br. abortus* by Bang and by Holth from uterine material and mummified fœtuses kept on ice for 7 to 8 months. McFadyean and Stockman (1909) record a similar experience, but failed to recover the organism at the end of 12 months. Under conditions of desiccation *Br. abortus* will die out in about 3 days.

Distribution of Br. melitensis and Br. paramelitensis.

Outside man. Br. melitensis is strictly parasitic and is found outside the body of its human or animal host only in places or on articles contaminated with the infective secretions or excretions. Active cases of undulant fever, ambulant cases, and infected goats and other animals excrete the brucella intermittently in the urine. The organism was found in considerable numbers in the soil and dust of Malta, particularly in the neighbourhood of goat-stables and markets. There are no records of a water-borne epidemic, despite the ability of the organism to survive in a potable water for long periods. The vast majority of infections in man are caused by the ingestion of fresh goats' milk, but it is possible that soil and fomites contaminated with urine play a minor part in the dissemination of infection, usually by direct inoculation through a breach of the skin surface. Nicolle and Conseil (1909) report the spontaneous infection of guinea-pigs kept in a goat-stable in Tunis probably attributable to contamination of food with dirt infected from urine. Goats' milk products, such as cheese or ice-cream, may contain the brucella in large numbers, and an additional danger from cheese is due to the common practice of ' freshening up ' the appearance of the cheese by immersing it in fresh goats' milk. The practice of ' wet milking ' is as universal among goatherds as among more civilized dairymen, and the spread of infection from goat to goat must in some part be attributed to this practice, the organism being inoculated from the hands of the milker into the numerous cuts and abrasions always present on the udders of native goats. The flesh of goats is a source of danger to slaughtermen and to those who handle the meat in a raw state.

The existence of the brucella in the stomachs of mosquitoes, *Culex pipiens* and *Stegomyia fasciata,* was established by the Royal Commission, but it was found that even if the organism was taken into the stomach it only remained there four days, and was then voided alive in the excreta. Fleas, bugs and blood-sucking flies were investigated with negative results, and it is not considered probable that insect vectors play any definite part in the dissemination of undulant fever.

Distribution in man. In an infected human being the brucella has a predilection for the spleen and lymphatic tissue and is present intermittently in the blood-stream. Elimination of the organism in the urine has been detected between the sixteenth and two hundred and forty-ninth day of the disease ; the excretion is intermittent and is not necessarily coincident with bacteriæmia. The number of cases which excrete the organism in the urine is probably higher than the figure of 10 per cent. given by the Royal Commission. The practical difficulty of isolating the brucella from the fæces has prohibited any extensive investigation in the subject ; Eyre (1908) isolated the organism from a case *post mortem,* but of 86 cases examined during life by the Royal Commission none was positive. Elimination in the milk has been reported. The brucella was not detected in the skin, breath, sweat or sputum of any of the cases examined by the Royal Commission.

Carriers. Ambulant and apyrexial cases are fairly common. Shaw (1906) found that 22 out of 525 Maltese dockers, all of whom were doing full work, gave evidence of infection ; 9 of these cases contained the organism either in the blood, or urine or both.

Distribution in animals. The Maltese goat is the animal showing the highest incidence of natural infection. Owing to its milking value it has been freely exported, and infection has passed from it to other goats and to cattle, ewes, mares, pigs, dogs and cats. In the Maltese goat, the brucella is present intermittently in the blood and urine and with marked constancy in the mammary lymphatics, mammary glands and milk. In other animals, including previously uninfected goats, the organism may be found intermittently in the blood and urine, and with considerable frequency in the lymphatic system, genital passages and milk ; aborted material is commonly infected.

Pathogenic Action of Br. melitensis and Br. paramelitensis.

The ability of *Br. melitensis* to produce disease and particularly symptoms of disease is greatly dependent on the species of animal receiving the infection. Thus, on the one hand, infection in man is manifested by marked febrile reaction, enlargement of the spleen and lymphatic glands, and a tendency to an inflammatory reaction in the joints, peripheral nerves, testicle and many other organs ; and, on the other hand, the infection in the Maltese goat is practically symptomless, the animal being merely a passive carrier, unless highly infected, in poor condition or suffering from some additional disease. Monkeys are the only experimental animals in which the typical undulant fever of man can be reproduced. Guinea-pigs, rabbits, mice and rats are susceptible to infection, the nature of the disease produced being dependent on the dose given and the virulence of the strain. Dogs and cats are sometimes chronic carriers, but are somewhat resistant to experimental infection. Ewes and heifers have been found to be naturally infected when living in close association with goats ; Evans (1923[1]) has produced abortion in a pregnant heifer six weeks after the intravenous inoculation of one agar slope of *Br. melitensis*. The question of the natural infection of cattle and pigs with *Br. melitensis* involves much careful work in distinguishing the organism from *Br. abortus*. Provided that an animal is susceptible to infection any exudate, material or fluid which contains the organism will bring about the infection. The route of administration is immaterial, be it by oral feeding, subcutaneous, intraperitoneal, intravenous, intratesticular, intramammary, intra-ovarian, intracerebral or intrathecal inoculation or by scarifying the skin or cornea.

ANALYSIS OF PATHOGENIC ACTION.

Br. melitensis produces no demonstrable exotoxin, and the major part of its pathogenic action must be regarded as due to endotoxins liberated from the dead organisms. Seven-day old broth cultures or agar slope cultures emulsified in saline killed by heat and filtered, comprising the various forms of ' melitine ', produce a definite dermal reaction when inoculated into infected human beings. It is evident, therefore, that infected subjects are sensitized to the toxic bacterial products as the result of their infection. The reaction to ' melitine ' is analogous to the tuberculin reaction of Koch. It has been found that cultures killed by 2 to 4 hours' contact with chloride of gold are less toxic than those killed by heating at 60° C.

The mortality from the disease is, in the average healthy adult, only about 2 per cent., and recovery is usual, unless there is some added complication such as renal disease, tuberculosis or other cachectic condition rendering the patient unfit to stand a long and exhausting illness. The relapses and recrudescences in man are probably due to a lighting up of small foci in the spleen, giving rise to a secondary bacteriæmia. The febrile reaction in man appears to assist in the elimination of the infection. On the other hand, in the Maltese goat, guinea-pig and other animals in which there is practically no fever, the infection is probably permanent, or, at least, of relatively longer duration. In such animals the toxic effects are purely local; they occur wherever there are infective foci, and result in mild chronic inflammation, which sometimes, as in guinea-pigs, gives rise to a definite histological picture.

Virulence was shown by Durham (1898) to be exalted as the result of intracerebral inoculation in an experimental animal, whilst it is well-known that frequent subcultivation renders a virulent strain almost saprophytic.

PATHOGENIC ACTION IN GOATS.

The susceptibility of the Maltese goat is shown by the high incidence of natural infection, and has been confirmed experimentally by the ease and constancy with which an infection may be produced either by subcutaneous or intravenous inoculation, or by the oral administration of living cultures. The dose required to produce infection is extremely small. Nevertheless, the reaction of the Maltese goat to infection is almost negligible, and, except in advanced cases and highly infected animals, there are usually no symptoms and no characteristic post-mortem lesions; during life infection may be proved by the cultivation of the brucella from the milk, urine or blood, and may be suspected by the appearance of agglutinins in the blood or milk.

The natural and experimental disease in the goat have been investigated by Zammit (1907 and 1922), Burnet (1923[1]), Burnet and Anderson (1924[1]) and Vincent (1922).

The result of intravenous inoculation with living brucella is a shortlived bacteriæmia, the organism being recoverable from the blood for varying periods up to a week. The infection rapidly becomes localized to the liver, spleen and lymphatic glands, whence secondary bacteriæmia arises from time to time. The udder and lymphatic system of the mammary gland become infected late in the experimental disease, but here the persistence of the infection is probably permanent. Animals which go 'dry' are found to be still infected when they again become pregnant. Zammit (1907) describes the case of an experimentally infected goat in which the milk contained no brucella, though it was examined frequently for a period of 18 months; the animal became dry and was impregnated, with the result that 3 days after kidding the organism appeared in the milk—2 years after the original inoculation. Foci of infection in the kidney and elimination of the organism in the urine are not so common in the goat as in man. In the goat the organism ceases to appear in the urine long before it disappears from the milk. In male goats the seminal vesicles and testicles not uncommonly harbour it.

In the naturally infected animal the disease probably runs approximately the same course. Bacteriæmia is rarely detected and urinary elimination is very intermittent. The main foci of the infection are the udder and the mammary lymphatic system of the female goat, and here a mild inflammatory reaction may be demonstrated, but there is no characteristic histological picture. Burnet and Anderson (1924²) state that mastitis is the essential lesion in goats, though clinical mastitis is in most cases due to secondary infection with pyogenic cocci. Only advanced cases exhibit any signs, and such signs are confined to alterations in the milk, which may be watery, thin, mucoid or blood-stained ; some few animals waste.

It is the opinion of Zammit (1907 and 1922), and Burnet and Anderson (1924¹), though denied by Vincent (1922), that neither an experimentally nor a naturally infected Maltese goat ever completely eliminates the infection.

Infection in the Maltese goat has been endemic for centuries, and the resistance of these animals is undoubtedly assisted by an inherited immunity ; abortion in the Maltese goat is not a common occurrence, though it is regarded as significant of a probable infection. But in what are described as ' unprotected ' goats, that is goats that have not the advantage of an inherited immunity, abortion is common, and the aborted material is highly infectious. After one or two abortions such goats develop some immunity, and thereafter become passive carriers like the Maltese goat. An epidemic of abortion may be expected to follow the introduction of an infected Maltese goat into an ' unprotected ' flock.

The most probable methods of infection from goat to goat are by contamination of food with urine, by direct inoculation into scratches and abrasions on the udder as the result of ' wet milking ', by flies which convey infected droplets of milk to scratches, to the mouth of the animal or to food, and by inoculation into the penis of the male when covering the female.

PATHOLOGY IN MAN.

The vast majority of human infections are due to the ingestion of infected goats' milk or goats'-milk products. After an average incubation period of 14 days the infection is shown by a febrile reaction, stubborn constipation and splenic enlargement. The onset of the fever corresponds with the appearance of the brucella in the blood-stream, and the organism is said to be most easily cultivated from samples of blood taken in the early afternoon. Successful blood cultures can usually be made at most of the febrile periods of the disease. The initial bacteriæmia is followed by the settlement of the organism in the spleen and lymphatic glands, particularly those of the mesentery, and in the liver, gall-bladder and kidneys.

In undulant fever there is a constant intoxication due to the presence of the brucella. The organism may apparently flourish in the internal organs for years, rapidly increasing at any favourable opportunity, presumably when vitality is lowered, and giving rise to fresh attacks of pyrexia.

Agglutinins make their appearance about the fifth day and often rise to a titre of several thousands. Though as a general rule a high titre indicates a favourable prognosis, this is not invariably the case, and, indeed, the titre shows such variation from case to case that care has to be exercised in relying on the agglutination reaction alone for making a diagnosis. Complement-fixing bodies are also found.

Throughout the course of the disease the brucella is excreted intermittently in the urine, and probably in the fæces, whilst it has also been found in the milk of lactating women by Burnet and Conseil (1925) and others. There is usually a fairly marked secondary anæmia and, invariably, a lymphocytosis, relative or absolute.

As the result of the toxæmia there is a tendency to inflammation in many organs ; arthritis, neuritis, epididymitis and orchitis are common ; hepatitis with jaundice, mild nephritis with albuminuria, bronchitis, serous pleurisy or peritonitis may occur ; purpura and hæmorrhages are found in severe cases ; endocarditis, possibly primary, but more probably an exacerbation of an old lesion, has been recorded ; meningitis, encephalitis, mastitis and ovaritis are rare. Abortion has been attributed to *melitensis* infection.

It is the exception for any effusion to become purulent, though suppuration is more frequent in paramelitensis infections.

In the tissues, *Br. melitensis* produces local irritation which may be followed by fibrosis, and sometimes by the formation of a cold abscess. Abscesses have been most frequently described as arising over the costochondral joints, and they may contain the organism in pure culture. Suppurative orchitis is rare.

Post mortem, the most constant finding is an enlarged and congested spleen which averages about 20 oz. in acute cases and 14 oz. in chronic cases ; weights up to 44 oz. have been recorded. The spleen is soft and sometimes diffluent ; the malpighian bodies are enlarged and there is considerable round-celled infiltration ; the organism is scattered throughout the substance of the organ in large numbers, and can almost invariably be cultivated from all fatal cases. The alimentary tract, kidneys and liver show congestion with cloudy swelling and necrotic foci. The mesenteric and other lymphatic glands may be enlarged ; the brucella may usually be cultivated from the former and from the kidneys.

Toxic myocarditis is a common cause of death and Bassett-Smith (1906[2]) records a case in which there was malignant endocarditis with infarction of the spleen and kidneys. Scott and Saphir (1928), in recording a fatal case of endocarditis, express the opinion that such infections are usually superposed on old rheumatic lesions.

Direct inoculation causes a high incidence of the disease in laboratory workers, whilst cases occur from time to time among the nursing staffs from handling infected urine or clothes contaminated with urine. Transference by mosquito bite and by dust has been shown to be feasible, but neither of these methods plays any material part in the incidence of human infections. Infections in goatherds and in cheesemakers by direct inoculation from milk, and in slaughtermen from handling infected goats' meat, are common. Early observers noted that there was no propagation by direct contagion from man to man or from bed to bed.

PATHOGENICITY TO GUINEA-PIGS.

Though it has been known for many years that *Br. melitensis* is pathogenic to the guinea-pig it is only in the last decade that characteristic histological lesions in the animal have been consistently reported. As a general rule when using reasonable doses of a living culture the febrile reaction is small, but though there is no undulant fever the organism shows the same tendency to linger in the spleen, lymphoid tissue and bone-marrow as in the case of the human disease.

The pathogenicity of a culture depends greatly on age, subcultivation, and on the dose given. Death within a few days may result from the intraperitoneal inoculation of a large dose of a recently isolated culture. Khaled (1921) found that three-quarters of an agar slope inoculated intraperitoneally killed a 240 gm. guinea-pig in 48 hours; the resulting peritonitis is serous rather than purulent. Smaller doses or less virulent strains, though causing little obvious change in the general health of the animal, nevertheless produce chronic granulomatous lesions in most of the organs, lesions which resemble closely the so-called '*abortus* disease' described by Fabyan (1912) as occurring after the intraperitoneal injection of *Br. abortus*. The granulomata appear as small nodules, sometimes only microscopic, consisting of epithelioid necrotic areas with occasional giant cells; they somewhat resemble tuberculous granulomata and are found most constantly in the spleen, lymph glands, lung, liver and testicle, but may be present in almost any part of the body except the voluntary muscles.

It is evident that by a nice gradation of dosage and taking due account of the route of inoculation there can be produced, in an average healthy batch of guinea-pigs, a result varying from death within a few days to a chronic generalized granulomatous condition from which the animal may eventually recover altogether. The type of disease produced, whether acute or chronic, is dependent on dosage and virulence rather than the route of inoculation.

Granulomatous lesions have been reported by Meyer, Fleischner and Shaw (1919) as the result of intratesticular injections with *Br. melitensis*, by Jaffé (1922), from subcutaneous, intraperitoneal and intratesticular inoculations, and by Kolesnikoff (1925) after the oral administration of living cultures.

Burnet (1922[1]) found that 20 per cent. of inoculated guinea-pigs developed arthritis, osteomyelitis and periarticular inflammation and that the organism is invariably recoverable from the marrow of the long bones.

Meyer, Fleischner and Shaw (1922) remark on the great variability in the individual susceptibility and this fact has been confirmed by Burnet's (1923[1]) comparative experiments when inoculating a series of animals intravenously with 100 millions of living brucellas.

Theobald Smith (1926[2]) has criticized much of the work of others on the ground that they used old strains and too large doses ; recently isolated strains were found to be much more virulent, and he considers that in the early phases the granulomata resulting from inoculations with *Br. melitensis* are distinguishable from '*abortus* disease' on account of the more violent nature of the lesions, there being with *Br. melitensis* far more necrosis, polymorphonuclear infiltration and a tendency to suppurate. Confirming the results and opinions of Bassett-Smith (1921), he found that *Paramelitensis* infections are more liable to suppurate than infections with *Br. melitensis* or *Br. abortus*.

Burnet (1925[1]) noted that second inoculations with living brucella produce in guinea-pigs a local reaction analogous to the tuberculin reaction and characterized by abscess formation. The same author found that guinea-pigs were more susceptible to the toxins contained in 'melitine' than to actual microbe bodies.

Evidence of the Responsibility of the Brucella Group for the Production of Undulant Fever in Man.

BRUCELLA MELITENSIS AND *BRUCELLA PARAMELITENSIS.*

The relationships of *Brucella melitensis* and *Brucella paramelitensis* to undulant fever was established by Bruce (1888) and Hughes (1893) as already stated.

BRUCELLA ABORTUS.

Since Evans (1918) reported the close morphological, cultural and serological similarities of *Br. abortus* of bovine origin to *Br. melitensis* of caprine and human origin there has been much controversy as to whether *Br. abortus*, so commonly found in cattle, is capable of producing undulant fever in man. At the outset it should be noted that the term '*Brucella abortus*' is commonly used to refer to the organism associated with infectious abortion in cattle. Nevertheless, a similar organism is found in other species of animals, particularly swine and horses, and it is necessary to state clearly the original source of any strain of *Br. abortus* when discussing its properties.

The problem as to whether all members of the brucella group are capable of producing undulant fever in man involves first an examination of the evidence for regarding all strains of *Br. abortus* as identical with *Br. melitensis*, and secondly a critical survey of the evidence for human infections resulting from sources other than caprine. The literature on the subject has been greatly confused and much of it rendered valueless by the inclusion of cases of ' *abortus* fever ' in which due care has not been taken to exclude a caprine source of infection, or to exclude a bovine infection from cattle which themselves had been infected primarily from goats. In many cases evidence for ' *abortus* ' infections has been adduced on a simple agglutination test without sufficient control absorption tests, whilst in experimental work fallacious deductions as to relative pathogenicity have resulted from the use of old and much-cultivated strains.

Evidence for the Identity of Br. melitensis *and* Br. abortus.

Morphology, culture and serology. The majority of observers agree that the two organisms present recognizable morphological and cultural differences, provided freshly isolated strains are studied. The characteristic features are described in the section on morphology and cultivation. Serological tests show that *Br. melitensis* and *Br. abortus* are closely related and that the brucella group as a whole is composed of a large number of serological races, which are, in many cases, so closely allied as to be distinguishable only by the most elaborate technique. The extensive investigations of Evans (1923[1] and 1925[1]) with 68 strains of brucella from human and animal sources showed that there were at least 9 serological groups, and that whereas an absorption test will frequently identify *Br. melitensis* it will not prove definitely that a strain is *Br. abortus*. Modifications of the agglutination test, such as the thermostability of agglutinins, &c., have as yet proved unsatisfactory in differentiating the brucellas. In the present state of knowledge, serological differentiation can only be effected with any degree of certainty, and not always then, by experienced workers and with adequate controls.

Experimental pathogenicity. Fleischner, Vecki, Shaw and Meyer (1921) studied the comparative virulence of *Br. melitensis* and *Br. abortus* (bovine) for monkeys by means of feeding experiments. They found that *Br. melitensis* was approximately one thousand times more infective than *Br. abortus* (bovine), and concluded that monkeys cannot be infected by the alimentary canal with relatively small doses of *Br. abortus* (bovine), even when feeding is continued for 30 days. They are some of the few observers who have attempted passage experiments to exalt the virulence of *Br. abortus* (bovine) ; by passage through goats, injecting the organism into the udder of the goat, they concluded that the virulence of *Br. abortus* (bovine), as tested by feeding experiments on monkeys, was increased. The difficulty of infecting macacus monkeys with *Br. abortus* (bovine) has been confirmed by Burnet (1922[2] and 1924[2]). On the other hand, *Br. abortus* (bovine) has been found by Meyer, Shaw and Fleischner (1922) and Burnet (1922[2]) to be distinctly more infective for guinea-pigs, for whereas *Br. abortus* (bovine) produces disease with great constancy, *Br. melitensis* does not always do so.

Though it appears from the experimental work that *Br. abortus* (bovine) is more invasive to guinea-pigs and less to monkeys than is *Br. melitensis*, yet it is not so certain that much of the comparative work has not been vitiated by the use of old strains of *Br. melitensis* and by the use of massive doses. Theobald Smith (1926[2]) states that the use of large doses tends to cause an acute condition in guinea-pigs, which obscures the characteristic differential features produced in these animals by *Br. abortus* (bovine) and *Br. melitensis* respectively. The value of the guinea-pig for differentiating *abortus* and *melitensis* strains has not been definitely confirmed.

A comparison of the experimental work with *Br. abortus* (bovine) in heifers and *Br. melitensis* in goats, and vice versa, shows that the pathology of the two infections is essentially the same. It has been shown that in virgin heifers inoculated with *Br. abortus* (bovine) the organism became localized in the mammary glands, whilst Burnet and Anderson (1924[1]) noted that after the intravenous inoculation of goats with *Br. melitensis* the organisms quickly disappeared from the blood-stream and became localized in the udder. Furthermore, Evans (1923[2]) produced abortion in a pregnant heifer by the intravenous inoculation of living *Br. melitensis*, whilst Edwards (1921) states that in goats, free from *melitensis* infection, abortion results from inoculation with *Br. abortus* (bovine).

Experimental or accidental infection in human beings. The high degree of infectiousness of *Br. melitensis* for laboratory workers is such a byword that almost any bacteriologist who works with freshly isolated strains may expect to contract undulant fever. On the other hand, laboratory infections with *Br. abortus* (bovine) are practically unknown : Huddleson (1926) has reported three cases. Furthermore, Nicolle, Burnet and Conseil (1923) failed to produce any illness in five human beings after inoculating them subcutaneously with large doses of living *Br. abortus* (bovine). It is probable, however, that the strains used were entirely saprophytic.

Natural infections in animals. The original caprine strains of *Br. melitensis* and *Br. paramelitensis*, so obviously infective to man, produce very little illness and no marked pathological lesions in the goats of Malta, Tunis and Algiers. On the other hand, in flocks of goats where infection has not been established for centuries, so-called ' unprotected flocks ', such as may be found in Switzerland, France, Mexico, Texas and some parts of Italy, a high proportion of abortion occurs. Ewes may be affected in a similar manner. Associated with epidemics of abortion in goats are frequently found outbreaks of undulant fever in man such as have been reported by the Sedallians (1924) in Lussan, and by Holt and Reynolds (1925) in Mexico. These caprine strains link up with the *Br. abortus* of bovine and porcine origin which are so definitely sexotropic, producing as they do a placental disease with a high proportion of abortions. The organisms are excreted in the milk and urine and are highly infective from animal to animal.

Cayrel (1926) records the isolation of typical *Br. melitensis* from the milk of cows in contact with goats and it is probable that the natural infection of cattle with caprine strains is not infrequent. In ' unprotected ' herds of goats or cattle the first effect of brucella infection is abortion; in any animal, after one or two abortions, immunity is developed and thereafter the organism, be it the *abortus* or *melitensis* variety, appears to settle in the mammary glands and to be excreted passively in the milk.

Cross-immunization. On account of the poor protection afforded by prophylactic inoculation very little work on this subject has been possible. Khaled (1921 and 1924) claims to have immunized monkeys by the intravenous inoculation of heat-killed cultures of *Br. abortus* (bovine), so that subsequent intravenous inoculation with living *Br. melitensis* failed to infect. The same observer states that the inoculation of goats with living *Br. abortus* (bovine) protects against infection with *Br. melitensis.*

Summary and conclusions. Whereas typical and freshly isolated strains of *Br. melitensis* and *Br. abortus* may be differentiated by morphological and cultural characters it is not always possible to do so in every case, and particularly with much-cultivated strains. No serological test will as yet identify with certainty all strains of brucella one from another, or if the test indicates which type is being dealt with it will give very little indication as to the probable pathogenicity for human beings. In animals lower than monkeys natural infection with any strain of Brucella is characterized essentially by abortion ; after the development of immunity, such as is found in Maltese goats, the animal, be it cow, goat, ewe or sow, becomes merely a passive carrier excreting the infecting organism in the urine and milk.

The identification of *Br. abortus* from human sources has been reviewed by Duncan (1928). Despite the close resemblances between all the brucellas there is such evident difference between the pathogenicity for man of the obviously virulent Mediterranean caprine *Br. melitensis* and the worldwide *Br. abortus* of infectious abortion in cattle that they cannot be said to be identical in the matter of virulence. It is possible that further work, by passage experiments, may show that the property of virulence for human beings can be exalted in the case of bovine strains, but in the present state of knowledge no laboratory test is sufficiently sensitive to indicate what is the factor which makes one strain pathogenic for man and another strain not.

It would seem that the brucella group consists of organisms evolved from a common parent which have developed grades of virulence, maybe as the result of sojourn in different species of animals, and that the differences in pathogenicity have become more emphasized than have the specific antigenic powers.

Evidence for the Ability of Br. abortus *to produce Undulant Fever in Man.*

Following upon the sporadic case of undulant fever reported by Keefer (1924), which was identified by Evans as due to a brucella of the *abortus* variety, attention has been directed to determining whether other cases of undulant fever may be traced to a bovine or porcine source of infection. The problem is rendered important by the fact that *Br. abortus* is found in large numbers in a high proportion of samples of cows' milk. In England, Kennedy (1914) and, more recently, Wilson and Nutt (1926) have found that upwards of 10 per cent. of single milks contain *Br. abortus.* In America, Schroeder and Cotton (1911), Evans (1916) and Huddleson (1922), and, in Germany, Zwick and Krage (1913) have all confirmed the frequency of the finding. It is evident, therefore, that there is ample opportunity for human infections to arise.

Geographical distribution. Whereas infectious abortion in cattle is of world-wide distribution and is very prevalent in temperate climates, the occurrence of undulant fever in man is mainly confined to tropical and subtropical climates. It is a striking fact that in France the areas where infectious abortion is epidemic are complementary to those where undulant fever is found. In the British Isles infectious abortion is common, but undulant fever of bovine origin has as yet only been suggested in a few reported cases. In North America there are definite endemic centres of undulant fever in Mexico, Texas and Arizona, but in these places goats are largely used for milking purposes ; abortion in the goats is common, and the strain of brucella has been identified by Evans as corresponding closely to Maltese strains. Bevan (1922) pointed out that in Southern Rhodesia there were occurring cases of undulant fever in man which were corresponding with outbreaks of infectious abortion in cattle. In some parts of Italy a similar state of affairs has been recorded. Assuming that *Br. abortus* (bovine) is able to cause undulant fever in man it would appear that there is considerable variation in the disease-producing power of the various strains distributed throughout the world. The most definitely pathogenic appear to be the Rhodesian strains.

Presence of antibodies for Br. abortus *in the blood of human beings.* A number of observers have investigated the sera of apparently normal persons for the presence of agglutinins, complement-fixers, or both, for *Br. abortus* (bovine). Nicolle and Pratt (1915), Sedgwick and Larsen (1913), Evans (1924) and Harrison and Wilson (1928), have all demonstrated agglutinins at varying titres in the blood of apparently normal persons. Kristensen (1928), in Copenhagen, has cultivated *Br. abortus* from the blood of several persons in whom low-titre agglutinins were discovered. Cooledge (1916[1]) brought evidence to show that *abortus*-infected milk contains also *abortus* antibodies, and that the latter can be made to appear in the blood of human beings by feeding ; he considers that the antibodies found by other observers are the result of passive absorption rather than active infection. Hull and Black (1927), in Illinois, where there are no goats, found 11 positive agglutination tests out of 69 sera. It would appear, therefore, that agglutinins for *Br. abortus* are present, sometimes in high titre, in the sera of a high proportion of human beings who do not show and who never have shown any evidence of undulant fever. As to whether the phenomenon is due to active immunization without symptoms of disease, to passive absorption or merely to non-specific effect is difficult to determine. It is well known that the agglutination test often fails to detect disease in clinically and culturally proven cases of undulant fever, and that a diagnosis made on agglutinins alone is frequently unreliable. In these circumstances it would be injudicious to attach undue importance to the finding of agglutinins for a group of organisms so variable in agglutinogenic power.

Recorded cases of undulant fever attributed to Br. abortus. In reviewing these cases it is necessary to remember that *Br. abortus* is found also in swine, and that porcine strains have been found to be far more invasive and infective than bovine strains. Secondly, many of the recorded cases show that there was a great chance of infection by direct inoculation from handling the genital organs, fœtus, placenta or membranes of an aborted cow or sow. In such circumstances it would be unwise to attribute the infection to the ingestion of cows' milk.

Cases from North America include those reviewed by Evans (1927), those reported by Gage and Gregory (1926), Huddleson (1926), Belyea (1927), and Sensenich and Giordano (1928). They include several of porcine origin and three of laboratory infection (Huddleson). The majority of infections have been of low grade, without relapses or recrudescences, and the organism has in most cases been isolated from the blood or urine or both.

In Rhodesia, Bevan (1922) recorded several cases of undulant fever coinciding with epidemics of abortion in cattle. He noted that the majority of cases occurred in males, subjects who would naturally be in close contact with aborting animals, and who would be exposed to risk of inoculation from handling cattle or soil infected by cattle. The same author (1926) records the occurrence of cases in patients who had had no contact with cattle, and in whom the probable source of infection was cows' milk. Duncan (1924) records a case in a Rhodesian butcher which he identified by absorption tests as due to a brucella of the *abortus* group. Orpen (1924) states that the Rhodesian strain of *Br. abortus* is serologically quite distinct from European strains of *Br. melitensis* and *Br. paramelitensis*, but is identical with the European *Br. abortus*. The Rhodesian strains of *Br. abortus*, however, have no need of additional CO_2 for their cultivation ; in this respect they resemble the strains isolated from the cattle themselves. The distribution of bovine strains of *Br. abortus* in Rhodesia is by no means simple, for there is a native strain, and a strain present in the pedigree cattle imported from England. Furthermore, Gow (1922) reviewed the question of goat infections in South Africa, and concluded that the previously free indigenous Boer goat had become infected owing to interbreeding with imported Angora and Swiss goats. It is evident, therefore, that though circumstantial evidence points to the probability that bovine strains of *Br. abortus* are responsible for the undulant fever of Southern Rhodesia, it is difficult to make the proof absolute in view of the numerous strains of brucella present in the various types of animals. At the present day it is believed that the Rhodesian strains of *Br. abortus* are virulent for human beings, and, indeed, are the most virulent of the various strains throughout the world.

Of cases occurring in Italy, the majority are reviewed by Cayrel (1926), and it is noteworthy that many of them (Ponticaccia; 1925 ; Tinti, 1925 ; Ficai and Alessandrini, 1925 ; and others) have occurred in men in attendance on cows at the time of abortion. It is stated by some that undulant fever, attributed to bovine infection, is more common in spring than at other times of the year, and this season is the time of maximum abortion in cows.

In the British Isles, cases diagnosed on the clinical history and a simple agglutination test have been reported by Byam (1918), Bamforth (1927), Wordley (1927), Manson-Bahr (1927[1]), Thompson (1928), Charles and Warren (1929) and Tod (1929).

Conclusions. In spite of the infrequency of laboratory human infection, of the failure of Burnet to produce undulant fever in human volunteers, and the relative non-pathogenicity of *Br. abortus* (bovine) to monkeys, it must be admitted on clinical and laboratory evidence that a fever in man comparable to undulant fever is sometimes caused by bovine strains of *Br. abortus*, and that these infections are most common in Rhodesia. At the same time, in view of the wide distribution of infectious abortion in cattle and the remarkable infrequency of associated undulant fever in man, the pathogenicity of the majority of strains of *Br. abortus* (bovine) to man must be low. On the one hand, there is the Rhodesian strain of evident pathogenicity and on the other hand the strains, such as those found in the British Isles, to which as yet only a few debatable cases of human infection have been attributed. Records of cases show that a high proportion of human cases have occurred in people who have had an opportunity of direct inoculation from the handling of infected animals or material, and even in Rhodesia it would appear that milk as a vehicle of infection is not in any way as dangerous as the milk of a Maltese goat excreting *Br. melitensis*. Bevan (1926) suggested that, though infection by milk may fail, yet inoculation of the blood-stream by a biting insect might succeed. The entomology of Rhodesia is favourable to such a means of propagation, and transmission of *Br. abortus* by insect-bite would appear to merit investigation. The majority of observers agree that porcine strains of *Br. abortus* resemble most closely the typical *Br. melitensis* both in virulence and as shown by laboratory tests.

In eliminating a caprine source of infection, the evidence is invariably confused by the existence of cross infections or double infections of cattle with caprine strains of *Br. melitensis*, in that the Maltese goat, on account of its high milking reputation has, at one time or another, been much exported. Thus, Evans (1925[2]) has found that the Mexican caprine strains are identical serologically with the Maltese strains, and in Mexico epidemics of undulant fever occur.

Present knowledge indicates that the genus Brucella consists of a number of species characterized by differences in virulence and pathogenicity rather than by differences in form, culture or antigenic specificity. The group forms a remarkable example of variation in invasive and disease-producing power according to the animal species receiving the infection. On the whole the majority of the lower animals react to a brucella infection, whatever the strain, by abortion ; eventually they cease to show any clinical evidence of infection and thereafter become passive carriers. Man and monkeys discriminate between strains, and respond, if at all, with a febrile reaction which eventually eliminates the infection altogether but does not produce a permanent immunity. Abortion in human beings is not commonly associated with undulant fever, but it should be noted that the production of abortion in a human being is notoriously more difficult than in an animal. As yet no satisfactory laboratory test has been invented, apart from the inoculation of monkeys, for differentiating those brucellas which are pathogenic to man from those which are not. Much work is required to determine and compare the pathogenicity to laboratory animals of the strains proved to be pathogenic to human beings, and to determine any factors which might lead to an exaltation in the virulence of strains now regarded as non-pathogenic.

Resistance of Man and Animals to Br. melitensis and Br. paramelitensis.

Human beings and animals possess but little natural immunity to infection with *Br. melitensis* and *Br. paramelitensis*, whilst the size of dose which will cause infection is extremely small. In man, the immunity resulting from active infection is so variable that it is of no value in estimating the probability of resisting a second infection ; passive carriers are not uncommon. In lower animals, the immunity from active infection is well marked so far as symptoms are concerned, but the majority become passive carriers for long periods, if not for life. Centuries of endemic infection in Maltese goats have created in these animals an inherited immunity to the signs of active infection ; abortion in Maltese goats is uncommon though a high proportion of animals are infected. Burnet and Conseil (1925) record a case of undulant fever in a lactating woman, and though *Br. melitensis* was recovered from the milk, yet the suckled infant did not develop the disease.

Infection with *Br. melitensis* does not protect against infection with *Br. paramelitensis* and vice versa, and, though Khaled (1924) claims to have immunized monkeys to *Br. melitensis* by injection of *Br. abortus*, yet the whole question of active immunity to the Brucella group is too uncertain to warrant an opinion as to the value, either of group or of specific immunization.

The Serological Reactions of the Brucellas.

AGGLUTINATION REACTIONS.

The agglutinogenic response to infections, naturally acquired or artificially produced, by the brucellas, or to the injection of killed cultures of these organisms, varies greatly with the individual subject. In man there may be a total absence of agglutinins in the blood in cases of well-established undulant fever, and the blood may remain free of agglutinins throughout the whole course of the disease. Burnet (1924[1]) estimates that the agglutination test gives a negative result in 20 per cent. of cases of undulant fever, but, on the other hand, agglutination titres of 1 : 4,000 or 1 : 5,000 are frequently met with. Variations, such as a sudden fall in the agglutination curve followed by a gradual rise to about the former level, often occur during the course of an infection, and the prozone phenomenon, affecting especially the lower titres, is exhibited frequently by the sera for this group.

The Serological Grouping of the Brucellas.

Judged by the variations in the results of absorbing agglutinating sera with different types of the genus, the agglutinogenic structure of the brucellas seems to comprise several elements, many of which are common to all types. It follows, therefore, that the simple agglutination test cannot serve as a means of distinguishing the types from one another. It is true to some extent that *Br. paramelitensis* stands out from the other types in its agglutination reactions, and this is in part due to its relative poverty in agglutinogenic elements.

To arrive at any system of serological grouping of the races it is necessary to employ the test of absorption of agglutinins, and by this means the brucellas may be arranged into a number of more or less ill-defined groups.

The most comprehensive system of grouping put forward up to the present is that of Evans (1925[2]), which is based on the examination of 68 strains from human, bovine, caprine, porcine and equine sources. She accepted as the criteria of group identity the capacity of the organisms to absorb to an equal degree from a heterologous serum and to exhaust a serum homologous to one of them. Reciprocal absorptions were carried out in some cases. The 68 strains were arranged into 8 groups, 3 of which contained only 1 strain each. The 5 groups remaining were designated by Evans as follows: Group III, *Br. abortus* (30 strains); Group V, *Br. para-abortus* (7 strains); Group VI, *Br. melitensis A* (12 strains); Group VII, *Br. melitensis B* (7 strains); and Group VIII, *Br. paramelitensis* (6 strains). The difference existing between these groups are in most cases very slight, sometimes amounting only to that between a strain and its corresponding substrain. The grouping is not related to the animal sources, but it seems to have some bearing upon the geographical distribution of the strains. Thus, Groups V, VII and VIII contained only strains of Mediterranean origin from human and caprine sources. Group VI contained 7 strains of United States origin from human, bovine and equine sources, and 5 from Europe. Group III included 28 strains from the United States from bovine and porcine sources and 5 from Europe. Altogether the series included only 5 bovine and no porcine strains from sources outside the United States, and as important centres of undulant fever, such as India, South Africa, Palestine and Peru were not represented, the number of groups cannot be regarded as complete.

Non-specific Agglutination by the Serum of Man and Animals.

Burnet and Lagoanère (1924) and others have shown that even in the presence of established infection by *Br. melitensis* the serum of man and some of the lower animals may be wholly deficient in agglutinins for the infecting organisms, or, even if agglutinins be present, flocculation may occur only with very low dilutions of the serum. On the other hand, non-specific agglutination of the brucellas occurs occasionally with the serum of normal individuals and perhaps more frequently with that of persons suffering from enteric fever, tuberculosis, kala-azar, tularæmia and certain other diseases. These non-specific reactions seem to depend not only on certain properties of the sera concerned, but also on an intrinsic tendency to non-specific agglutination on the part of some strains of the micro-organisms. This applies particularly in the case of *Br. paramelitensis.*

Nègre and Raynaud (1911) examining the sera of 39 persons, presumed not to have suffered from undulant fever, found 22 of them to agglutinate *Br. melitensis*, and in 8 cases the reaction occurred in titres up to 1 : 100. These alleged non-specific reactions should be regarded with suspicion when they occur in areas where undulant fever is endemic ; Shaw (1905) investigated the blood of 525 apparently healthy dock labourers employed in Malta, and got agglutination of *Br. melitensis* in 79 cases, and in 22 of these the reaction was marked. On further examination of these 22 cases *Br. melitensis* was recovered from 10. The possibility not only of recovered cases but of ambulatory cases must not be overlooked.

In recent years the United States have furnished many reports of agglutination of the brucellas by the blood of persons living in areas where undulant fever is believed not to exist. Such instances have been found chiefly in the routine examination of blood samples sent from hospitals and dispensaries for Wassermann and Widal tests. Evans, in Washington (1925^2), got 59 positive reactions amongst 500 sera tested, and in 16 the titre of agglutination reached 1 : 10 or higher. Hull and Black (1927) found 11 positive out of 69 sera, 5 reaching a titre of 1 : 200 ; Arnold and Miller (1927), 14 positive out of 200 sera ; Clark (1927), 6 positive out of 110 sera ; Young, in Michigan, 11 positive out of 110 sera, with 5 reaching a titre of 1 : 300 ; and there are many reports of similar findings. The tendency in the United States is to regard these reactions not as non-specific but as evidence of casual infection by *Br. abortus.* In England, Bassett-Smith (1907^2) found only 2 out of 100 hospital sera to agglutinate *Br. melitensis*, and these in low titre only.

To avoid the error arising from non-specific reactions, the adoption of a minimum diagnostic titre of agglutination has been suggested; Cole (1913) recommended a minimum titre of 1 : 80; Kolle and Hetsch (1911), 1 : 100; d'Oelnitz and Liotard (1924), 1 : 100 to 1 : 150; Hull and Black (1927), 1 : 50; Bassett-Smith (1906[1]), 1 : 30; and Niçolle (1905), 1 : 10. It is clear that if the higher figures be accepted as the safe minimum, a large proportion of true specific reactions will be disregarded.

The Thermal Stability of Agglutinins.

To render the agglutination test more specific, Nègre and Raynaud (1911) recommended heating the serum under investigation to 56° C. for 30 minutes before use, as it was supposed that at this temperature the non-specific agglutinins were inactivated while the specific agglutinins remained unimpaired. This procedure received general acceptance, and is still extensively used in connection with the agglutination test in undulant fever. It has been shown, however, by several observers that the destructive action of a temperature of 56° C. is exercised not only on the non-specific agglutinins, but, perhaps even to a greater extent, also on some of the specific agglutinins. Canale (1925) in an investigation of 600 sera found that a serum agglutinating *Br. melitensis* up to a titre of 1 : 600 might be completely inactivated at this temperature. Although instances such as this seem to be exceptional and in general experience many agglutinating sera are found to be quite unaffected by a temperature of 56° C., it is advisable to conduct agglutination tests at temperatures of from 37° C. to 40° C.

The different agglutinins for the brucella group vary in their resistance to high temperatures, but, on the whole, these elements in a serum which affect chiefly the *Br. abortus* type are the most resistant. This feature forms the basis of a test used by Ficai and Alessandrini (1925) to distinguish *Br. melitensis* from *Br. abortus*. Assuming that most of the agglutinins for *Br. melitensis* are inactivated by a temperature of 65° C. but may resist 60° C., while those affecting *Br. abortus* can withstand 65° C. and sometimes even 70° C., but are destroyed at 75° C., their method consisted in diluting the serum to 1 : 50 with distilled water and heating to 65° C. for 30 minutes, after which, it is asserted, only agglutinins specific for *Br. abortus* would remain active. The value of the test is doubtful and the results obtained by Ficai and Alessandrini have not received confirmation in the work of many other investigators. In this connection, Tapia and de Nicholas (1926) suggest that the thermal stability of the agglutinins is related to the animal source of the serum rather than to the type of bacterium used in its production.

Factors affecting the Agglutination of the Brucellas.

Influence of the culture medium on agglutinability. There is little doubt that the early luxuriant growths obtained on some of the richer media give suspensions of higher agglutinability than those prepared from the slower growth on relatively poor media. Izar (1926) states that the prolonged propagation of a strain on a rich medium, not only raises agglutinability, but also renders it less specific.

While there is no clear evidence that agglutinability is materially influenced by the reaction of the culture medium, it has been suggested by Domingo (1924) that it shows an increase with the diminishing acidity of the medium from pH 6·6 to pH 8·8.

The brucellas, like some other species, when cultivated in a liquid medium containing small quantities of active agglutinating serum, become less agglutinable with each subculture, until after about twelve passages they may be inagglutinable by the serum.

Influence of bile on agglutinability. Cultivation of the brucellas in the presence of bile or commercial bile-salts, or the treatment of suspensions of the organisms with either, exalts agglutinability. To achieve this, Domingo (1924) recommends the addition of one volume of a thick suspension of the organism to nine volumes of sterile filtered ox-bile. The mixture is allowed to stand, with 3 or 4 shakings, for 24 hours, and the organisms are then collected by centrifuging at high speed, and re-suspended in isotonic salt solution to the optimum density. The suspension is freed from clumps by filtration through a layer of filter-paper, and 0·2 per cent. of formol is added as a fixing agent and preservative. The formol also improves agglutinability. This treatment is said to render some inagglutinable strains agglutinable, to exalt greatly the agglutinability of strains of low agglutinability and to render the reaction more specific by destroying the susceptibility to non-specific agglutination. In the case of strains of normally good agglutinability the effects of bile treatment are comparatively slight, and any increase in the specificity of their serological affinities appears not to affect their intimate connections with other types of the genus. In place of filtered natural bile, a solution of 7 per cent. commercial bile-salt may be used.

Flocculation by Lactic Acid.

Lactic acid, more than any other agent, exhibits differences in its flocculating effects on different strains of the genus, and it has been claimed by Vercellana and Zanzucchi (1926) that these differences are distinctive of different species, and constitute a means of separating *Br. melitensis* from *Br. abortus*. For identification they recommend the following test. A 1 per cent. solution of lactic acid is carried through a series of graduated dilutions with distilled water in small test-tubes. Then to each tube in the series is added an equal volume of bacterial suspension in distilled water, and a control, with distilled water in place of the acid solution, is included. The mixtures are incubated at 37° C. for 30 minutes and then examined. Suspensions of *Br. melitensis* are said to show good flocculation, while those of *Br. abortus* are either unaffected or give only weak reactions. Vercellana and Zanzucchi regard agglutination in titres of 1 : 300 or higher as pointing to *Br. melitensis*, and absence of agglutination or agglutination in low titres only, to *Br. abortus*. There is no doubt that *Br. melitensis* strains generally show a somewhat greater susceptibility to flocculation by this acid than do *Br. abortus* strains, but, as other investigators have found, the differences are not sufficiently constant to make this a specific test.

Flocculation by Heat.

Burnet (1925[2]) has shown that suspensions of *Br. paramelitensis* in isotonic salt solution, when heated to 90° C., flocculate rapidly. After flocculation the suspensions may be re-established by shaking, but flocculation will recur in a few hours if the tubes are allowed to stand on the bench. The quality of the flocculation and the velocity of the reaction appear to be correlated with the predominance of other characters more or less peculiar to the *paramelitensis* type; such are the commoner tendency to spontaneous flocculation and to non-specific flocculation, and weak antigenic properties shown by the inability of the organism to elicit a high-titre agglutinating serum when injected into a rabbit. Complete flocculation with large flocculi forming within a few minutes is associated with the pure *paramelitensis* types, while slower reactions taking up to about 30 minutes with small flocculi and incomplete flocculation occur with the less typical forms of *Br. paramelitensis*. Young cultures flocculate more completely than older ones, and the intensity of the reaction diminishes with the age of the culture until eventually it disappears.

Neither *Br. melitensis* nor *Br. abortus* exhibits any tendency to flocculation by heat, but *Br. melitensis* grown in broth which has already supported a culture of *Br. paramelitensis* may acquire this property transiently.

OTHER SEROLOGICAL REACTIONS.

Fixation of Complement.

The test of complement fixation has been employed in the diagnosis of undulant fever as alternative to or confirmatory of the agglutination test. It has also been employed in the detection of cattle infections by *Br. abortus*. Various antigens have been used, but the most suitable are carbolized and centrifuged autolysates of the organism (Mohler and Traum, 1911), extracts obtained by shaking heat-killed cultures and centrifuging or filtering (Mohler and Eichhorn, 1911), solutions in isotonic saline of alcoholic precipitates of filtrates of autolysed cultures (Holth, 1909), or fresh heat-killed suspensions of the organisms. The value of the test in the diagnosis of infection is seriously affected by the fact that the serum of normal people may yield a positive reaction; Larsen and Sedgwick (1913) found 17 per cent. of the sera from 425 normal children to react positively, while the sera from 42 breast-fed infants who had never drunk cows' milk gave negative results. Cooledge (1916[1]), as a result of similar experiments, found that the reaction depended upon the ingestion of milk from infected cows, and concluded that it was due to a partial passive immunity derived from the ingestion of *Br. abortus* antibodies present in the milk.

The Precipitin Test.

The precipitin test, using as precipitinogen filtered extracts of cultures, possesses no advantage over the complement-fixation test and is even less trustworthy.

IMMUNIZATION OF MAN AND ANIMALS FOR TREATMENT AND PREVENTION OF INFECTIONS WITH *BR. MELITENSIS* AND *BR. PARAMELITENSIS.*

Man.

The tendency of undulant fever to natural remission or cure makes difficult an accurate estimate of the true value of any remedy, and accounts for the conflicting opinions as to the value of vaccine therapy. Whereas one case will apparently respond well to vaccine therapy, another will be unaltered or even made worse, despite the use of either a stock or an autogenous, a sensitized, an iodized or any form of vaccine. Bassett-Smith (1907[1 & 2]), from a long experience, believed that vaccines shortened the course of the disease and were of value in preventing relapses. Severe types of fever are not benefited as much as the mild and intermittent cases or as much as those in whom the symptoms are mainly local. The most favourable time for administration is after the crest of a pyrexial wave has been passed, and the treatment should be omitted at the onset of another bout of fever. An average initial dose is 200 millions, and if the injections are made at 7- to 10-day intervals and the maximum doses do not exceed 400 millions, then the local and general reactions are usually slight.

Khaled (1924), Auricchio (1924) and others have used *abortus* vaccines and claim that a higher dosage can be employed with a better effect and with less reaction. Domingo (1925) reports success with an oral vaccine. Chloroform- or ether-killed vaccines are stated to be superior to heat-killed preparations. Serum from immunized animals or from convalescents has no appreciable effect on the course of the disease. Many authorities state that the intradermic injection of melitine or abortine has a therapeutic as well as a diagnostic value, and it seems probable that the therapeutic value of melitine preparations would merit investigation.

Prophylactic inoculation is commonly employed for immunizing laboratory workers, but it is questionable whether any marked immunity results. It is probable that prophylactic vaccination shortens the duration and severity of the disease without giving much protection against actual infection. The dosage employed is two injections of 1,000 millions with a 7- to 10-day interval.

Animals.

The immunization of goats with Vincent's vaccine is discussed on p. 426.

Practical Diagnosis of Infections with Br. melitensis and Br. paramelitensis.

IN MAN.

Laboratory diagnosis of brucella infections is made by the isolation of the organism from the blood, urine, milk or tissues, by the demonstration of antibodies, particularly agglutinins, and by the presence or absence of cutaneous sensitivity.

Cultivation from the Blood.

Br. melitensis has been recovered from the peripheral blood in 68 per cent. of 103 Maltese cases recorded by Shaw (1905²), and in a similar proportion of 45 cases examined by Gilmour (1906). It has been found present as early as the second day of illness (Gilmour, 1906), and it may still be recovered from cases of 9 or 10 months' duration. Although the organisms may be found in the peripheral blood during afebrile periods their presence in the circulation is inconstant, and they are more frequently recovered during a febrile exacerbation, especially if the fever has lasted for a few days. Shaw (1905¹) found that the afternoon was a more favourable time for obtaining successful blood cultures than the morning. He could find no correlation between the degree of pyrexia and the intensity of bacteriæmia. When the organism is present, it may be recovered from a very small quantity of blood ; Shaw has recovered it from 4 c.mm. of blood, and Horrocks and Kennedy (1905) cultivated it from the blood in the stomachs of mosquitoes which had fed on patients. The average weight of such a blood feed would be about 3 mgm., and as many as 100 colonies developed on a plate spread with the stomach contents of a single mosquito.

Methods of cultivation.

Blood should be collected from a vein at the most favourable time, under aseptic conditions, in a sterilized syringe, according to the usual technique for hæmoculture. It may then be planted in liquid or on solid media.

Liquid media. Trypsinized broth prepared according to the formula of Douglas and Colebrook (1916) is a useful medium. The presence of trypsin greatly favours the growth of *Br. melitensis*, but its antithrombic properties and its action in neutralizing the antibacterial factors in fresh blood, are not so necessary as when cultivating the salmonellas, for *Br. melitensis* will grow fairly well in the clot ; a fact which is made use of in Tizzani's method of cultivation. Therefore, in making cultures directly into simple, nutrient broth, a relatively large volume of the medium is unnecessary, and successful cultures may be obtained when the blood is added in the proportion of one volume to about eight volumes of the medium. On the other hand, great dispersion of the organisms such as occurs when the blood is highly diluted, as in adding 1 c.cm. to a litre of broth, seems not to prejudice the chances of growth of *Br. melitensis* as it might those of *Br. abortus*, in which some co-operation amongst the organisms is necessary to start growth. One of the best liquid media for hæmoculture is Stafseth's liver infusion broth (see p. 393). About 3 or 4 c.cm. of blood from a vein sown in 100 c.cm. of the medium will usually give a growth of the organism in 3 to 4 days, but failure should not be accepted until the flasks have remained sterile for 2 weeks.

From the primary growth subcultures may be made on to Fildes's peptic-digest-blood-agar medium, liver-infusion-agar or glucose-agar, and in the case of the richer media a good growth will be found usually in 24 hours.

In Tizzani's method, referred to above, the patient's blood is distributed in quantities of a few c.cm. in about four sterilized test-tubes. In these tubes it is allowed to clot, and the expressed serum is removed and replaced by sterile broth, which in turn is changed every 24 hours for a few days. It is assumed that by the removal of all the serum with the contained antibacterial factors, the organism is permitted to grow in the clot with more certainty.

Solid media. The organism may be recovered sometimes by spreading the blood directly on the surface of an agar plate, but the probability of success is greatly improved by using a richer medium such as nutrose-glucose-agar or liver-infusion-agar with or without the addition of gentian violet.

Cultivation from the Urine.

The presence of *Br. melitensis* in the urine is usually intermittent; daily examination may reveal its presence once only in a period of more than a month, or it may make a second transient appearance after an interval of 1 or 2 weeks. On the other hand, occasionally, it has been found present daily, with odd intermissions of a single day, for a month or more. Kennedy (1905) states that it may appear as 'a sudden enormous gush which stops as suddenly as it appears' or as 'a long-continued excretion in small quantities'. The presence of the organism in the urine is not necessarily influenced by the state of bacteriæmia, and a considerable degree of bacteriæmia is consistent with the continued absence of the organism from the urine. From Kennedy's observations it appears that the excretion of the organism in the urine is related to certain phases of the disease, especially to 'early convalescence or the last stages of the fever', and that the onset of another fever wave tends to stop the excretion. The organism may appear in the urine at intervals for some little time after defervescence; the longest period recorded by Kennedy is 33 days. In prolonged infections it may still be excreted, and it has been recovered from the urine after 10 months' illness.

In attempting to obtain primary cultures of *Br. melitensis* from the urine it is desirable to plate out directly on some suitable solid medium, as, owing to its slow growth and its frequent association in the urine with other organisms equally resistant, the ordinary enrichment methods using bile, antiseptic dyes, &c., in a liquid medium cannot be applied.

A catheter specimen or a ' mid-stream ' specimen should be collected with the usual precautions to ensure asepsis. For plating out urine, the medium employed by the Mediterranean Fever Commission (1905–7) was glucose-nutrose-agar (containing 1 per cent. each of nutrose and glucose) tinted with litmus, which made it possible to distinguish colonies of the common glucose fermenters from those of *Br. melitensis*, which has no action on this sugar. The quantity of urine spread on each plate should be about 0·25 c.cm. for a 4-inch plate, increasing proportionately with plates of larger size. The urine is spread as a thin film over the whole surface of the plate, and when it has soaked in sufficiently the plate may be inverted. Colonies of *Br. melitensis* do not, as a rule, appear earlier than the fourth or fifth day at 37° C., but a practised worker can usually identify them, when well developed, by their pearly appearance. Suspicious-looking colonies should be picked off, subcultured and examined in the usual way, or a microscopic agglutination test may be performed, using a high-titre serum and a suspension prepared directly from the colony.

If enrichment is necessary, or as a routine method, the liver infusion agar with 0·01 per cent. gentian violet may be used as the plating medium.

Cultivation from Tissue.

Splenic puncture was originally used by Bruce and is sometimes successful when other methods fail. The procedure is rarely justifiable owing to the softness of the organ and the danger of a fatal hæmorrhage.

Post mortem, *Br. melitensis* is recovered most frequently from the lymphatic glands, especially the mesenteric glands, the spleen and the liver. It may also be present in the circulating fluids, the bone-marrow, the testis and rarely the kidney. From the solid tissues the organism may best be cultivated by tearing out, under aseptic conditions, tiny fragments, which should be rubbed well over the surface of a gentian-violet-liver-agar plate or some other suitable medium.

Demonstration of Antibodies.

Apart from the special features relating to thermostability, chemical stability and specificity of the agglutinins described on pp. 412–417, a simple agglutination test is of considerable value in diagnosis. Blood-serum is most frequently employed but agglutinins may also be demonstrated in milk and inflammatory effusions. Owing to the infectivity of the organism the test emulsion should be either phenolized or killed by heating to 60° C. for 30 minutes ; alternatively, an emulsion of the less infective *Br. abortus* may be used. It is advisable to employ a polyvalent emulsion of *Br. melitensis* made from strains not liable to auto-agglutinate and not agglutinable by non-specific factors in low titre. A simultaneous test with *Br. paramelitensis* should always be performed. The density of the emulsion has a bearing on the titre of the reaction, and can, to some extent, influence prozone phenomena and non-specific results. Bassett-Smith recommended a density corresponding to 10 times the strength of the typhoid emulsions of Dreyer (1909), whilst Evans (1923[1]) arrives at approximately the same density by adjusting to 20,000 parts per million of the silica standard of the American Public Health Association and thereafter diluting 1 in 1,000 with saline. As a routine measure, Bassett-Smith (1921) recommended the following procedure: Take sufficient serum and divide it in 2 parts. Heat one in the water-bath for half an hour at 56° C. Use a 48-hour culture of a reliable strain to make the emulsion. Put up 3 dilutions of the heated and unheated serum at 1 in 40, 1 in 100 and 1 in 400, using the macroscopic technique. Incubate at 37° C. for 2 hours, and then stand at room temperature until the result is read at the end of 12 to 24 hours. Controls with normal and specific sera should be put up at the same time. Heating the serum tends to prevent non-specific results, but it also reduces the agglutination titre, and may entirely obliterate a reaction in a serum of low titre.

It is estimated by Burnet (1924^1) that the agglutination test fails to detect 20 per cent. of active cases of undulant fever. Proven cases may fail to develop agglutinins throughout the whole course of the disease, and, on the other hand, titres as high as 1 in 5,000 are frequently encountered. The reaction may be demonstrated from the fifth day of the disease onwards. Any titre from 1 in 30 upwards may be taken as adding weight to a clinical diagnosis and titres of 1 in 100 or more are practically diagnostic. The agglutination reaction is most liable to be unsatisfactory in markedly toxic and cachectic cases. The reaction is usually of low titre in carriers and may be absent altogether.

Cross-agglutination between *B. tularense* and *Br. melitensis* has been reported by Francis and Evans (1926). The serum from a tularæmia case will agglutinate both *B. tularense* and *Br. melitensis*, but Burnet (1927^2) states that the serum of a case of undulant fever will not agglutinate *B. tularense*. An absorption test readily differentiates the two organisms. Non-specific agglutination of *V. choleræ* by the sera of undulant fever patients has been recorded by Barra (1919). Low titre non-specific agglutination sometimes occurs with normal sera and has also been recorded with the sera of tuberculous patients ; Amabile (1924) investigated the sera of 86 tuberculous patients without detecting any agglutinins for the Brucella group. The value of a complement-fixation test is described on p. 416.

Cutaneous Sensitivity.

Cutaneous sensitivity was introduced as a diagnostic test for undulant fever by Burnet (1922^1). The preparation used by Burnet is the Chamberland filtrate of a month-old broth culture of *Br. melitensis ;* the efficiency of the preparation is unimpaired by adding phenol, and only slightly reduced by boiling for one minute. The intradermic injection of one drop of the filtrate causes redness and local œdema in about 7 hours increasing in 12 to 24 hours to a zone 4 to 6 mm. across with a sharply demarcated margin. Trenti (1923 and 1925) confirms Burnet's findings, but recommends a polyvalent preparation made from at least six strains of *Br. melitensis.*

Various preparations of ' melitine ' have been advocated. Fornaca and Bua-Fazio (1924) used a phenolized and heat-killed saline emulsion of a 6- to 8-day agar slant culture having a content of about 2 million organisms per c.cm. ; 0·5 c.cm. of the emulsion is injected intradermally and a control performed with phenolized saline. Fornaca's preparation results in a vesicle within an hour which increases in 16 hours to a red area with surrounding œdema and infiltration ; the reaction begins to decline in 24 to 48 hours and disappears in 5 to 7 days. Canale (1925) recommends a dried preparation whilst Bua (1927) used ' abortine ', a saline emulsion of *Br. abortus,* and found it as effective as melitine. Abortine will not differentiate undulant fever caused by *Br. abortus* from that caused by *Br. melitensis.*

General experience shows that melitine or abortine reactions are specific for active brucella infections in human beings though mild reactions have been reported in typhoidal and tuberculous patients. The reaction is of value in detecting carriers. The duration of the reaction is not accurately known ; it may be elicited from the twelfth to the thirty-fifth day of the disease and onwards, and in convalescents three months or more after apparent cure.

Diagnosis in Animals.

Cultivation from the Naturally Infected Goat.

In the dissemination of *Br. melitensis* by the carrier goat the chief vehicle is the milk, and it was estimated at the time of the Mediterranean Fever Commission's investigations (1905–7) that 10 per cent. of the milch goats in Malta were excreting the organism in their milk. *Br. melitensis* may be excreted in the milk of the individual goat either continuously or intermittently. Thousands of organisms per c.cm. may be present on one day, and perhaps none within a few days.

Agglutination of *Br. melitensis* by the milk is sometimes taken as an indication of the probable presence of the organism in the milk, but when the excretion of the organism is intermittent the milk may show a marked agglutinating power for long periods when there are no organisms present. Occasionally the serum alone may show a high agglutinating titre in animals which apparently have never excreted the organism in their milk. A more trustworthy guide is the absence of agglutinins for *Br. melitensis* from both the blood and the milk, which is generally accepted as evidence against the probability of the organism being found in the milk.

Br. melitensis appears in the milk only after the goat has been infected for some time, in experimentally infected animals about two to four months after infection, and its presence is due probably to the mechanical flushing of the mammary gland, one of the last sites of localization of the organism in the animal.

Cultivation from goat's milk. The most suitable media for the plating out of milk are the glucose-nutrose-litmus agar or the enriching medium liver-infusion-gentian-violet agar. Although the number of the organisms which may be present in the milk, sometimes 2 or 3 thousand per c.cm., is sufficiently great to yield a large number of colonies from a plating of the whole milk, it is customary to centrifuge 5 c.cm. of milk at high speed, and spread a series of plates from the deposit and cream. When the milk is allowed to stand in cool surroundings for about 24 hours until the cream has separated naturally, it is found that nearly all of the organisms have been carried up with the cream, and a plating from the cream layer yields more than twice the number of colonies developed on plates spread from either the cream or the deposit of an equal volume of the original milk separated by centrifugalization. Plating from the cream separated slowly in the cold seems, therefore, to be the method of choice for all milks.

As the organisms present in the milk represent merely those flushed from the mammary ducts, it is sometimes a more convenient procedure, and necessary if the goat is ' dry ', to practise puncture of the udder and withdrawal of some of the matter contained in the lactiferous ducts.

Cultivation from the blood of the goat. Bacteriæmia in the goat is an intermittent condition, and occurs chiefly in the earlier stages of the infection, before strict localization of the organism has occurred. As a general rule, even when infection is evidenced by a high agglutination titre of the serum or the presence of *Br. melitensis* in the milk, it is an unusual experience to find the organism in the blood.

Cultivation from the urine of the goat. The appearance of *Br. melitensis* in the urine of the infected goat is very inconstant, much more so than in the case of man. When present, its isolation is rendered difficult by the presence of many contaminating organisms.

Culture of a brucella by inoculation of a susceptible animal. Amongst the smaller experimental animals, the guinea-pig presents a high degree of susceptibility to infection by the brucellas ; a minute dose of any type introduced into the peritoneum may cause a prolonged infection. Although the inoculation of the guinea-pig is employed chiefly for the recovery of *Br. abortus* from cows' milk, from which the organism may be difficult to isolate by other means, it may also be used for the recovery of *Br. melitensis* from the blood, urine, milk or tissues of man or animals.

The method employed is as follows : Quantities of about 1 to 2 c.cm. of blood, fresh or citrated, or 2 to 3 c.cm. of milk or urine may be injected intraperitoneally into a short series of guinea-pigs. In the case of blood clot or solid tissues, the material should be ground up under sterile conditions in a mortar in a few c.cm. of isotonic saline solution with the aid of sand. After sedimenting, the supernatant liquid is collected and injected as above. Between the fifth and seventh days, one of the guinea-pigs is selected and killed and its peritoneal cavity washed out with a few c.cm. of sterilized saline. The washings are sown on to suitable media, and the cultures followed up in the usual way. If the first guinea-pig yields nothing, the others in the series should be killed off at intervals from the third to the sixth week, and cultures attempted from the spleen, lymphatic glands and testes by rubbing fragments of these organs over the surface of liver-agar-gentian-violet medium.

The organism tends to disappear fairly rapidly from the peritoneum after the first week and to multiply in the spleen up to the fourth week, after which its numbers diminish, and eventually it may be found only in the lymphatic glands.

Before killing the inoculated animals, evidence pointing to infection may be found in the occurrence of a febrile reaction, slight loss of weight and, usually after the first week, the presence in the blood of agglutinins for the infecting organism.

Agglutination by Milk.

Although not so valuable a test as serum agglutination, milk agglutination may be resorted to in the absence of blood samples or to ascertain the probability of the organisms being excreted in the milk. For the test it is desirable to use a clear whey, and this may be obtained by clotting the milk with rennet ; any increase in the acidity of the whey should be corrected before preparing the test. The technique is then similar to that of serum agglutination. Zammit (1922) has devised a simple method in which whole milk is used. Drops or loopfuls of milk diluted with water or saline to 1 : 10 or 1 : 20 are distributed on glass slides, and each is mixed with a similar quantity of a thick suspension of *Br. melitensis.* The resulting mixtures, in which the milk is now diluted to 1 : 20 or 1 : 40, are taken up into short capillary tubes which are then sealed at both ends and placed upright on a bed of plasticine. The results are read on the following day, although the reaction is usually quite distinct in a few hours, and the naked-eye appearance of the precipitate is very characteristic.

Other Methods for Diagnosis in Goats.

Cutaneous sensitivity is very unreliable (Burnet, 1923^2, 1924^1). Aborted material may yield a positive culture. *Post mortem,* the mammary gland, the mammary lymphatic glands and the spleen are the most likely tissues to contain the organism.

Diagnosis in Other Animals.

Cultures should be made from aborted material and from milk, and the investigation should include the inoculation of a guinea-pig.

Chemotherapy of Infections with Br. melitensis and Br. paramelitensis.

In undulant fever, as in other bacteriæmias, the reported successes from intravenous treatment with chemicals have been few and by no means constant, whilst the general difficulty of finding a germicide active in the presence of serum has not been overcome. The arsenical preparations, novarsenobillon, galyl, &c., have been much used in Africa ; it is probable that the beneficial action is due as much to tonic effect as to bactericidal power. Flavine compounds have not given good clinical results, though *in vitro* tests by Izar and Mastrojeni (1927) showed that gonacrene and tripaflavine are actively bactericidal for *Br. melitensis*, in the presence of serum, at a dilution of 1 in 256,000. Mercurochrome (0·22 gm.) has been used with success by Gage and Gregory (1926), but is apt to cause severe general reactions. Ziemann (1921) reports favourably on doses of 2 to 4 c.cm. of 2 per cent. collargol, whilst Souleyre (1921) claims that 1 per cent. colloidal silver and colloidal gold are of great assistance in subacute cases ; the depressant action of the metals calls for simultaneous treatment with stimulants.

Autohæmotherapy and protein shock therapy have been used by Girbal (1923), Oddo (1925) and others ; 5 c.cm. of whole blood are injected intramuscularly. Other methods include the production of a fixation abscess with iodide of starch or turpentine ; abscesses so formed usually contain *Br. melitensis* in pure culture.

A true estimate of the value of chemotherapy is difficult to make owing to the tendency of the disease to natural remission or cure ; isolated successes have therefore little value as evidence. The majority of chemicals give the best results in subacute and non-toxic cases, and it may be inferred that much of the benefit can be attributed to shock and the resulting febrile reaction rather than to a specific action on the causal organism.

Prevention of Undulant Fever.

It is now unanimously accepted that the most common method of contracting infection is by the ingestion of goats' milk and milk products such as ice-cream, butter and cheese. It is a practical axiom, as the result of experience, that where goats are gathered in herds and are used for the general milk supply of the population there undulant fever is fairly sure to develop. Other domestic animals such as cows, sheep, mares, donkeys, dogs and pigs may become infected from goats, but these, with the exception of cows, rarely spread the infection, in that the milk of such animals is not commonly consumed. Human cases excrete the organism in the urine so that infection in attendants may be contracted by lack of hygiene. Direct inoculation in nurses, laboratory workers, goatherds and slaughtermen is responsible for a few cases in that the dose necessary to cause infection is extremely small.

Bearing in mind that the majority of infected goats are mere passive carriers and exhibit very few signs of an infection from which they probably never become free, and that the organism is easily destroyed by sunlight, antiseptics and heat, the necessary preventive measures are clearly indicated.

Goats.

1. The notification of all infections identified in goats and of any cases of abortion should be compulsory. Infected animals should be destroyed

to prevent dissemination of the infection. Treatment or isolation of infected animals is useless as they probably never eliminate the infection, but destruction may be limited to those animals containing the organism in the blood, milk or urine. The flesh of destroyed animals should not be used for food. Goats may be tested by culture of the blood, urine and milk, and by the testing of the serum and milk for agglutinins. Those animals giving a positive agglutination test should be kept under observation, but not destroyed unless excreting the organism. The intradermic test is not reliable in goats.

2. Non-infected goats should be protected by the use of Vincent's (1918) vaccine. This consists of the subcutaneous injection at 5 to 8 days' interval of 4,000 M of a polyvalent *melitensis* and *paramelitensis* ether-killed vaccine. Vincent's results have been confirmed in principle by Ascoli and Sanfilippo (1927), but vaccines have been found to be useless by Zammit (1922) and Burnet (1927[1]). The method merits further trial.

3. Attention should be directed to the hygiene of goat stables. These are frequently badly drained, highly contaminated with infected urine and swarming with flies.

4. Prevention of the introduction of fresh goats into a herd unless passed as free from infection. Ambulant herds are a great source of danger.

5. Education in hygienic methods of milking and in particular the stamping out of the process of ' wet milking '.

6. The proper disposal of infected goat carcasses. Slaughtermen should know that the animals are infectious and that the disease is easily contracted from handling the infected meat, which should not be used for food.

Man.

1. The thorough sterilization of all goats' milk and its products must be rigorously enforced. Boiling or pasteurization are equally effective. Milk should be sterilized before being made into cheese.

2. The urine and excreta of all cases should be thoroughly disinfected.

3. The danger of direct inoculation should be impressed upon all nurses, attendants, goatherds, slaughtermen and laboratory workers. In view of the occurrence of undulant fever associated with epidemic abortion in cattle, farm hands and veterinary surgeons should realize that aborted material may be infectious and particularly so in the case of swine abortion. In districts where undulant fever is associated with bovine abortion the latter disease should be made notifiable and the outbreak treated as in the case where goats are the source of infection.

4. In endemic centres latrines and urinals of any field force should be carefully supervised, as should be also the sanitary provision in mines.

5. Prophylactic vaccination is by no means reliable, and is far less important than hygienic measures, but should be given to those employed in milking goats and to laboratory workers. Two injections of 1,000 M are given subcutaneously at an interval of seven days.

Brucella abortus Infection in Cattle.

BY A. D. MCEWEN.

GENERAL.

When the pregnant female becomes infected with *Br. abortus*, the uterus is generally invaded and the animal is likely to abort or calve prematurely; but with some animals, despite a uterine infection, parturition will occur at the end of the normal period of gestation. When the uterus is infected the bacteria are usually also present in the udder and are secreted in the milk for periods of time ranging from a few weeks to several years. A small number of pregnant animals escape uterine infection despite the presence of bacteria in the udder; and again a few animals when exposed and infected (infection being indicated by the appearance of antibodies in the serum) exhibit a resistance which enables them to overcome the infection, with the result that *Br. abortus* is found neither in the uterus at parturition nor in the milk.

Animals which have aborted or calved prematurely, as a result of a uterine infection with *Br. abortus*, generally give birth to normal or apparently normal calves at subsequent pregnancies, although some of these animals may harbour *Br. abortus* in the uterus at the time of calving. A few animals, however, abort more than once. The symptoms of *Br. abortus* infection in the female are confined to those associated with the act of abortion or premature calving and are common to these disorders irrespective of their ætiology. A not infrequent sequel is retention of the fœtal membranes, which become infected with secondary micro-organisms and are disintegrated and gradually discharged, sometimes causing the reproductive functions temporarily or permanently to be interfered with. In the aborting animal the quantity of the milk yield is diminished.

When infection occurs in the male the lesion is an inflammation confined to the testicle and epididymis.

PATHOLOGY.

In the female the only known sites where *Br. abortus* can become established are the pregnant uterus, the mammary gland, and the lymphatic systems of these organs.

When the pregnant uterus and the fœtus are infected the most extensive lesions are found in the fœtal membranes. A number of the cotyledons present necrotic changes ranging from necrosis of a few villi to an extensive involvement of the whole cotyledon. The diseased cotyledons occasionally become detached from the maternal carunculæ, the carunculæ participating in the necrotic process. The interplacental portions of the chorion show areas of discoloration, opacity, œdema, thickening and leatherlike degeneration, and Theobald Smith (1919) has shown that the outermost cells of the diseased chorion are invaded by *Br. abortus*. These diseased cells may be shed and constitute, together with mucus, fibrin and leucocytes, the yellowish to brownish, purulent exudate found between the chorion and the uterine mucosa. Underneath the exudate the mucous membrane of the uterus is normal in appearance, though Sven Wall (1909) has demonstrated that microscopically a superficial necrosis is present.

In the extensively diseased fœtus the subcutaneous and muscular tissues are œdematous, and blood-tinged effusions are often present in the large serous cavities ; the lungs occasionally show areas of consolidation ; meconium is generally discharged into the amniotic fluid and may be swallowed by the fœtus, and the fourth stomach and the intestine contain a purulent-like material. In cases of generalized infection *Br. abortus* is recoverable from the different organs and body fluids. Lesions in the less extensively diseased fœtus are confined to the stomach, intestines and lungs, and *Br. abortus* is recoverable from these organs or from the stomach and intestinal contents only.

In rare instances the fœtus is not expelled but becomes mummified. Should the fœtus be expelled late in pregnancy it may be living and, in appearance, normal. Theobald Smith (1925) has shown that pneumonia in viable calves is sometimes due to *Br. abortus* infection acquired *in utero.*

The changes produced in the mammary gland are not extensive. Schroeder and Cotton (1911) drew attention to the possibility of small areas of induration in the mammary tissue being due to *Br. abortus* infection, and Runnells and Huddleson (1925) have demonstrated small inflammatory foci in mammary glands where *Br. abortus* was the only micro-organism present.

Invasion of the testicle and epididymis may produce extensive degeneration and necrotic changes in these organs.

MEANS BY WHICH INFECTION IS ACQUIRED.

The infected female is capable of disseminating *Br. abortus* by two routes ; one is from the uterus at, and for a few weeks after, abortion or parturition ; the other is by the elimination of bacteria in the milk. Of these the dissemination from the uterus is the most important, and constitutes the greatest danger, as bacteria are present in large numbers in the diseased fœtus, fœtal membranes, and uterine discharges, and readily contaminate the pasture, food, water and buildings, where, if conditions of moisture, temperature and light are suitable, the bacteria may remain viable for lengthy periods (p. 397).

As a rule the bacteria disappear from the uterus and uterine discharges a few weeks after abortion or calving. McFadyean and Stockman (1909) failed to find them in the uterine discharges after the sixth week from the date of the abortion ; however, they have been found after 60 days, and it is possible that in rare instances they are present for longer periods. Schroeder (1922) was unable to demonstrate *Br. abortus* in the non-pregnant uterus of animals inoculated intravenously with *Br. abortus* cultures, either at œstrum or between the œstral periods.

The disease is usually acquired by the ingestion of the bacteria. Bang (1906) demonstrated that infection could be produced by feeding the pregnant female with portions of diseased fœtal membranes, and McFadyean and Stockman (1909) established the fact that susceptible females were easily infected by being fed with material from an infected uterus, or with a culture. Subsequent work has confirmed these findings.

McFadyean and Stockman (1909) produced infection by inoculating the bacteria into the vagina, and McFadyean, Sheather and Minett (1913) succeeded in inducing a positive serological response in male animals by inoculating into the prepuce. It is, therefore, theoretically possible for the infection to be acquired by the bull during coitus, and for the bull mechanically to transmit infection from an infected to a healthy female. Schroeder and Cotton (1916) found that *Br. abortus* was recoverable from the ejaculated semen of an infected bull. Nevertheless, it has not been definitely proved that the bull is capable of transmitting the infection to the female during coitus. The readiness with which uninfected pregnant animals contract the disease, when they are introduced into a herd in which abortion disease is active, further indicates the relative unimportance of the possibility of infection being transmitted by coitus.

The infected mammary gland has not been incriminated as a direct source for the spread of the infection from animal to animal. Schroeder and Cotton (1916) were successful in producing abortion by inoculating *Br. abortus* into the teat canal, but there is insufficient evidence to support a supposition that *Br. abortus* may be transmitted from udder to udder on the hands of the milker or by milking machines ; and, indeed, pregnant non-lactating heifers are as susceptible to the infection as pregnant lactating cows, which would not be expected were infection spread through milking. Infected milk may be spilt and contaminate the pastures and food, &c., and Hayes and Barger (1922) found that calves fed on infected milk discharged viable *Br. abortus* in their fæces, but whether infection can be produced by the relatively small number of bacteria spread in these ways is very doubtful. Nevertheless, the infected udder must be regarded as a potential reservoir for the extensive dissemination of the bacteria, as bacteria harboured in the udder may spread to and infect the pregnant uterus and finally be discharged in great numbers.

Viable calves born with diseased fœtal membranes may discharge *Br. abortus* in the meconium, and so constitute a possible source for the spread of the infection.

Experimentally, infection can be brought about by instilling small quantities of culture into the conjunctival sac, by subcutaneous and by intravenous inoculation, the latter method being the most certain means of producing the disease.

SUSCEPTIBILITY.

Female calves are highly resistant to permanent infection both by the ingestion of *Br. abortus* and by subcutaneous inoculation with cultures of the bacteria, and Quinlan (1923) showed that calves which reacted positively to the serological test, when removed from their dams and kept free from further infection, gave no evidence of *Br. abortus* infection when they were bred. The insusceptibility is continued in the heifer up to the time when conception takes place, though probably in a lessened degree. With the onset of pregnancy a susceptible stage is reached and is maximal two or three months after pregnancy has been established. The commencement and continuance of lactation predisposes the udder to *Br. abortus* infection, but infection may occur in this organ when the animal is not pregnant. After sexual maturity has been attained the actual age of the animal would appear to play an unimportant part, uterine infection occurring probably as readily in the cow carrying its second or later calf as in the pregnant heifer.

In the male there is not the same sharp dividing line between a non-susceptible and a susceptible state, dependent upon the functional activity of the reproductive organs, as apparently exists in the female. Carpenter (1924) was able to recover *Br. abortus* from the epididymis of a young calf fed with artificially infected milk, and Seddon (1919) produced testicular lesions by the intravenous inoculation of *Br. abortus* culture into a three month's old calf. The incidence of infection in bulls, as indicated by the serological tests, has been shown to run a parallel course with the degree of infection in the females. Of those bulls reacting to the serological test the number presenting lesions from which *Br. abortus* is recoverable is probably small, and is estimated by Buck, Creech and Ladson (1919) to be approximately 11 per cent.

EPIZOOTIOLOGY.

When *Br. abortus* infection is first introduced into a herd a large number of animals may become infected, and should the animals be due to calve at about the same period, the disease may manifest itself by a more or less simultaneous appearance of a large number of abortions. The majority of animals abort only once, and during the following year there may be few or no abortions in the herd. When the animals are due to calve at different periods the disease may show itself by occasional abortions occurring over lengthy periods of time. Once infection has become established throughout a herd the disease tends to reach a quiescent stage, as far as the act of abortion is concerned, until such time as fresh uninfected susceptible animals are introduced or until the young heifers in the herd become pregnant, when there is grave danger of their becoming infected and aborting.

DIAGNOSIS.

Bacteriological examination. The occurrence of abortions or premature calvings with or without retention of the fœtal membranes is very suggestive of *Br. abortus* infection. A positive diagnosis can only be made with certainty by the demonstration of the bacteria, though the presence of specific antibodies in the serum is generally regarded as evidence that the abortions are due to *Br. abortus*. When bacteria are present in the uterine discharges in large numbers they may be demonstrated in suitably stained smears, and are seen as occurring in clumps or clusters, which are probably inclusions of the bacteria in shed chorionic epithelial cells, or leucocytes. Impression smears from the chorionic epithelium and sections of the chorion may reveal the characteristic cellular inclusions of *Br. abortus*.

Bacteria which are not demonstrable microscopically may be recovered from the uterine discharges, fœtus, or fœtal membranes by guinea-pig inoculation or by cultural means. The presence of bacteria in the milk may be verified in a similar manner.

With adequate technique cultural methods are probably as reliable as guinea-pig inoculations, but should the suspected material be heavily contaminated with extraneous micro-organisms, guinea-pig inoculation is the only possible method. The demonstration of specific agglutinins in the sera of inoculated guinea-pigs affords further proof.

Serological tests. Following *Br. abortus* infection, antibodies appear in the serum and in the milk, and are demonstrable by the agglutination test and the complement-fixation test. The results of these two tests are closely concordant, but on account of the greater simplicity of the agglutination test it is used almost exclusively for routine diagnosis. As a rule, antibodies are demonstrable in the serum a few days after infection occurs, but in a number of infected animals which abort or which contain *Br. abortus* in the uterus at the time of calving, antibodies may not be demonstrable until shortly before parturition. Huddleson, Torrey and Carlson (1928), in studying the agglutination reactions of the animals in an infected herd, found that 15 per cent. of the infected animals did not react until a day or a few days before aborting. A correlation between a maintained high titre of the serum and persistence of infection in the udder has been noted by Schroeder and Cotton (1924) ; Mathews (1926) and Huddleson, Torrey and Carlson (1928) have found that when the agglutination test shows a declining titre over considerable periods of time the animals have become free from the disease.

The majority of animals with specific antibodies in the serum show these in the milk, but Cooledge (1916^2) found that there was no constant relationship between the antibody content of the serum and of the milk. The serum may be positive and the milk negative, or the titre of the serum may be high while the titre of the milk is low. The concentration of antibodies in the different quarters of the same udder is also subject to variation. When bacteria are present in the milk antibodies are generally demonstrable, but exceptionable cases are recorded of the isolation of bacteria from the milk though agglutinins were absent both from the milk and from the serum.

The colostrum of an infected animal contains antibodies in high concentration. Calves born of infected mothers give a negative serological reaction at the time of birth, but Little and Orcutt (1923) showed that, after ingestion of colostrum, agglutinins and complement-binding antibodies rapidly appeared in the serum reaching a maximum concentration in five hours. These antibodies remain demonstrable in the serum up to about the seventh month ; thereafter, as a rule, they disappear despite the ingestion of *Br. abortus* in the milk. Calves fed on high-titre colostrum tend to remain reactors longer than those fed on low-titre colostrum.

Allergic reaction. McFadyean and Stockman (1909) first attempted to diagnose *Br. abortus* infection by the use of *abortin,* a material analogous to tuberculin. The abortin was inoculated subcutaneously and a positive reaction was judged on the basis of a definite thermal response. Consistent and satisfactory results were not obtained, and similar findings have been reported from Germany and the United States. An abortin containing the bacteria killed by heat was employed intradermally by Reichel and Harkins (1917), but results were less satisfactory than were those obtained by serological tests. Lately, Holtum (1928) reported favourably on the use of a similar diagnostic agent when a double intradermal inoculation was made.

Summary. It may be said that the demonstration of the bacteria is the only certain means of positively establishing a diagnosis ; that the agglutination and complement-fixation tests are most reliable methods for distinguishing animals which are infected or have been infected, and that when a positive serological reaction is associated with a history of abortion or premature calving it is very strong presumptive evidence that the disease was caused by *Br. abortus* infection ; that the diagnosis based on an allergic reaction is much less reliable than one founded on a serological test.

VACCINATION.

The observation that cows which have aborted seldom abort again led to the belief that immunity or resistance was produced, and this belief has been substantiated by the controlled experiments of Birch (1927), who caused pregnant animals to abort by feeding them with cultures of *Br. abortus,* and then showed that at the next pregnancy these animals were resistant to a fresh infection.

Early work by Bang (1906) with vaccines prepared from killed bacteria did not hold out encouraging prospects and the failure of subsequent workers to produce a satisfactory resistance by this means led to the abandonment of killed vaccines in favour of vaccination with living cultures. It has been found that inoculation of virulent living cultures of *Br. abortus* into non-pregnant animals two or more months before conception is attended with but slight risk of abortion, though Hart and Traum (1925) have shown that the subcutaneous inoculation of living vaccine into non-pregnant but lactating cows is capable of causing infection of the udder ; the possibility of infection spreading from that organ to the uterus during pregnancy cannot be disregarded.

Field experiments in England, Germany and the United States with living vaccine all demonstrate a lower abortion ratio amongst the vaccinated than among the control animals, whilst the carefully controlled vaccination experiments of Smith and Little (1923), Buck and Creech (1924), Hart and Traum (1925) and Lubbehusen, Fitch and Boyd (1926), where results were checked by bacteriological examinations of the contents and discharges of the uterus and of the milk, and by serological tests, all show a definite increased resistance and a lower degree of infection in the animals vaccinated with living cultures. The results of these experiments vary in their success, the most favourable being recorded by Hart and Traum. These differences may be due to some extent to variations in the susceptibility of the experimental animals ; some animals appear to be hypersensitive and repeatedly abort. Smith and Little (1923) believe that such animals do not benefit from vaccination, and that vaccination by failing to protect shows up those animals which are likely to become chronic aborters.

It has been shown that if heifers are vaccinated shortly before conception they may be protected as readily as lactating cows, and in the heifers there is little likelihood of the vaccine causing an udder infection. There would, therefore, appear to be no necessary relationship between the persistence of *Br. abortus* in the animal's body and an acquired resistance to a fresh infection ; Buck and Creech (1924) have brought forward evidence to indicate that animals which have aborted but do not harbour *Br. abortus* in the udder are more resistant to a fresh infection than are animals which have aborted and continue to secrete *Br. abortus* in the milk. As living vaccine, which is virulent to some degree, does not prevent abortion or infection of the uterus in all cases and may even be directly responsible for udder infection in cows, its use does not eliminate *Br. abortus* infection, though it may reduce the number of abortions in an infected herd and curtail the dissemination of the micro-organisms. The vaccine is, therefore, only used in herds already infected. In herds where the disease is actively manifested by abortions or premature calvings its use has not been shown to increase the percentage of animals eliminating *Br. abortus* from their udders.

Vaccination with living attenuated strains of *Br. abortus*, incapable of causing infection in the susceptible bovine, has been favourably reported on by Huddleson (1924), and is deserving of further study. The vaccine has not been found capable of influencing the course of the disease once infection is established. The experiments of Buck and Creech (1925) have shown that repeated inoculations of a heat-killed vaccine are quite ineffective in overcoming infection of the udder.

ERADICATION AND CONTROL.

The only means of effectually eradicating *Br. abortus* disease consists in the segregation of those animals reacting positively to the agglutination or complement-fixation test, these tests being applied at 30- to 60-day intervals to all animals of breeding age in the herd, and until such time as reacting animals do not occur. After this the test may be made at greater intervals in order to check a fresh appearance of the disease. At the same time separate stalls, capable of being satisfactorily disinfected, should be provided for parturition, and animals should not be released from these until they have been shown to give a negative serological reaction. Rigorous precautions must be taken to prevent the introduction of infection in freshly acquired animals. Pregnant females should not be permitted to enter the herd, but should be kept isolated until they have calved and reacted negatively to the serological test. Males and non-pregnant females should be isolated until they have given two consecutive negative reactions with a month's interval between the tests. The bulls in the non-infected herds should not be used for serving infected females. By exercising the measures of eradication and control indicated above, it has been repeatedly shown that *Br. abortus* can be entirely eliminated from herds and these herds maintained free from infection. The time required to eradicate the disease depends upon the extent of the infection in the herd and the thoroughness with which the eradication measures are enforced.

When circumstances do not permit the following of the eradication policy, efforts may be made to limit the spread of the disease by separating the positively from the negatively reacting animals as much as possible, and by the use of parturition stalls where animals, after calving, remain until the uterine discharges have ceased. Calves born of infected mothers may be removed when three or four months old and reared separately; after passing a negative serological test they may be included with the non-infected herd.

In infected herds where these measures are inapplicable vaccination with the living bacteria may be employed.

Brucella abortus Infection in Swine.

Br. abortus infection of swine has been reported from the United States and from Germany. The disease in these animals follows a course similar to that run by the disease in cattle. In a sow the pregnant uterus and mammary gland are predilection sites for the bacteria, and a uterine infection may cause the animal to abort. Schroeder and Cotton (1925) have described lesions in the ovary caused by *Br. abortus*, and in this respect the disease in swine differs from that of cattle. The predilection sites for the bacteria in the boar are the genital glands.

The disease may be diagnosed by the demonstration of the bacteria in aborted material and in the uterine discharges, and by the aid of the serological tests.

The discovery of *Br. abortus* as a cause of disease in swine raised the question of the infection being acquired by these animals from cattle, but investigation has not established any such relationship, and experimentally it is difficult to produce abortion in swine by feeding them with bacteria recovered from cattle; cattle appear to be much more resistant to infection with porcine strains than to infection with bovine strains.

The differentiation of bovine from porcine strains. Distinct differences in virulence are shown by bovine strains and porcine strains of *Br. abortus.* The porcine strains are possessed of greater invasive powers and cause a more acute disease in laboratory animals. Guinea-pigs infected with porcine strains frequently show abscessation in the orbital cavity and bones; lesions which are not produced by typical bovine strains.

In their biological characters the two types of strains also differ. The great majority of bovine strains require an increased CO_2 concentration of the atmosphere to produce surface growth in primary cultures, but with porcine strains this is not so. McAlpine and Slanetz (1928) found that bovine strains, accustomed to growing on artificial media, were stimulated in their growth by the addition of 5 to 10 per cent. of CO_2 to the atmosphere, while porcine strains under similar conditions were inhibited in their growth. Further, these authors showed that bovine strains were incapable of utilizing glucose, whilst porcine strains use considerable quantities of the carbohydrate; these differences in ability to utilize glucose are reflected in the nitrogenous metabolism of the cultures.

A variation in the growth-inhibitory action brought about by incorporating gentian violet in the medium has been used by Huddleson and Abell (1928) for distinguishing bovine from porcine strains. Concentrations of 1 : 50,000 to 1 : 100,000 inhibited, but did not completely prevent, the growth of bovine strains; but similar concentrations of the dye completely prevented the growth of porcine strains.

Serologically it is impossible to distinguish the one type from the other.

Exceptional strains of cattle origin fail to conform to all the foregoing characteristics of the bovine strains, but the recorded exceptions are few and it is probable that these strains were recovered from cattle infected with the porcine variety.

It may be concluded that two distinct varieties of *Br. abortus* are recognizable. These, though indistinguishable serologically, may be distinguished by differences in virulence and metabolic activity. These two varieties, with rare exceptions, originate from two separate sources: namely, cattle and swine, and permit the recognition of a definite bovine type and a definite porcine type of *Br. abortus.*

Brucella abortus Infection of Domestic Animals other than Cattle and Swine.

Br. abortus has been recorded as the cause of abortion in the mare, but would appear to be of very rare occurrence and little importance. Natural cases of infection are unknown in the other domestic animals.

REFERENCES.

AMABILE, L., 1924, *Riforma med.*, **40,** 217.

ARNOLD, L. & MILLER, W. E., 1927, *Proc. Soc. Exp. Biol.*, N.Y., **24,** 836.

ASCOLI, M. & SANFILIPPO, E., 1927, *J. Trop. Med.*, **30,** 19.

AURICCHIO, L., 1924, *Pediatria,* **32,** 57.

BAMFORTH, J., 1927, *Lancet,* Lond., i, 818.

BANG, B., 1897, *Z. Thiermed.*, **1,** 241 ; 1906, *J. Comp. Path.*, **19,** 191.

BASSETT-SMITH, P. W., 1906^1, *Rep. Mediterranean Fever Commission to Royal Society,* London, Pt. IV, 101 ; 1906^2, *Brit. Med. J.*, i, 313 ; 1907^1, *J. Hyg.*, Camb., **7,** 115 ; 1907^2, *J. Trop. Med.*, **10,** 169 ; 1921, *ibid.*, **24,** 53.

BELYEA, G. N., 1927, *J. Amer. Med. Ass.*, **88,** 1482.

BEVAN, L. E. W., 1922, *Trans. Soc. Trop. Med. Hyg.*, Lond., **15,** 215 ; 1926, *Proc. R. Soc. Med.* (Joint Discussion), **19,** 8.

BIRCH, R. R., 1927, *Cornell. Vet.*, **17,** 221.

BIRT, C. & LAMB, G., 1899, *Lancet,* Lond., ii, 701.

BRUCE, D., 1887, *Practitioner,* **39,** 161 ; 1888, *ibid.*, **40,** 241 ; 1893, *Ann. Inst. Pasteur,* **7,** 289.

BUA, F., 1927, *Policlinico,* **34,** 631.

BUCK, J. M. & CREECH, G. T.,1924, *J. Agric. Res.*, **28,** 607 ; 1925, *ibid.*, **31,** 663.

BUCK, J. M., CREECH, G. T. & LADSON, H. H., 1919, *J. Agric. Res.*, **17,** 239.

BURNET, E., 1922^1, *C.R. Acad. Sci.*, Paris, **174,** 421 and 973 ; 1922^2, *ibid.*, **175,** 790 ; 1923^1, *Arch. Inst. Pasteur,* Tunis, **12,** 213 ; 1923^2, *C.R. Acad. Sci.*, Paris, **177,** 414 ; 1924^1, *Arch. Inst. Pasteur,* Tunis, **13,** 1 and 5 ; 1924^2, *C.R. Acad. Sci.*, Paris, **178,** 154 ; 1925^1, *Arch. Inst. Pasteur,* Tunis, **14,** 62 ; 1925^2, *C.R. Acad. Sci.*, Paris, **180,** 2085 ; 1927^1, *ibid.*, **185,** 1627 ; 1927^2, *Arch. Inst. Pasteur,* Tunis, **16,** 90.

BURNET, E. & ANDERSON, C., 1924^1, *Arch. Inst. Pasteur,* Tunis, **13,** 7 ; 1924^2, *C.R. Acad. Sci.*, Paris, **178,** 428.

BURNET, E. & CONSEIL, E., 1925, *Arch. Inst. Pasteur,* Tunis, **14,** 121.

BURNET, E. & LAGOANÈRE, J. L., 1924, *Bull. Soc. Path. exotique,* **17,** 465.

BURRA, L. T., 1919, *Lancet,* Lond., i, 64.

BYAM, W., 1918, *Lancet,* Lond., i, 873.

CANALE, P., 1925, *Riv. clin. Med.*, **26,** 527 and 631.

CARPENTER, C. M., 1924, *Cornell. Vet.*, **14,** 16 ; 1926, *J. Infect. Dis.*, **39,** 220, cited from 1927, *Trop. Dis. Bull.*, **24,** 114.

CAYREL, A. M., 1926, *Paris méd.*, **16,** 141.

CHARLES, J. A. & WARREN, S. H., 1929, *Lancet*, Lond., i, 386.

CLARK, T., 1927, *Bull. Off. int. Hyg. publ.*, **19,** 1460.

COLE, 1913, cited from *Milit. Surg.*, **33,** 540.

COOLEDGE, L. H., 1916^1, *J. Med. Res.*, **34,** 459 ; 1916^2, *Tech. Bull.*, **33,** *Mich. Agric. Expt. Sta.*

D'OELSNITZ, M. & LIOTARD, M., 1924, *Bull. Soc. méd. Hôp. Paris,* **48,** 548.

DOMINGO, P., 1924, *C.R. Soc. Biol.*, Paris, **90,** 819 and 824 ; 1925, *Rev. Med. Barcelona,* 2 s., **4,** 240.

DOUGLAS, S. R. & COLEBROOK, L., 1916, *Lancet,* Lond., ii, 180.

DUNCAN, J. T., 1924, *Trans. Soc. Trop. Med. Hyg.*, Lond., **18,** 318 ; 1928, *ibid.*, **22,** 269.

DURHAM, H. E., 1898, *J. Path. Bact.*, **5,** 377.

DREYER, G., 1909, *J. Path. Bact.*, **13,** 331.

EDWARDS, J. T., 1921, *Vet. Rec.*, **33,** 721.

EVANS, A. C., 1916, *J. Infect. Dis.*, **18,** 437 ; 1918, *ibid.*, **22,** 576 ; 1923^1, *Publ. Hlth. Rep.*, Wash., **38,** 1948 ; 1923^2, *ibid.*, **38,** 825 ; 1924, *ibid.*, **39,** 501 ; 1925^1, *Amer. J. Trop. Med.*, **5,** 419 ; 1925^2, *Bull. U.S. Hyg. Lab.*, No. 143 ; 1927, *J. Amer. Med. Ass.*, **88,** 630.

EYRE, J. W. H., 1908, *Lancet,* Lond., i, 174 ; 1925, *Proc. R. Soc. Med.* (Joint Disc.), **19,** 1.

FABYAN, M., 1912, *J. Med. Res.*, **26,** 441.

FEUSIER, M. L. & MEYER, K. F., 1920, *J. Infect. Dis.*, **27,** 185.

FICAI, G. & ALESSANDRINI, A., 1925, *Policlinico* (sez. prat.), **32,** 113.

FILDES, P., 1920, *Brit. J. Exp. Path.*, **1,** 129.

Fleischner, E. C., Vecki, M., Shaw, E. B. & Meyer, K. F., 1921, *J. Infect. Dis.*, **29**, 663.

Fornaca, L. & Bua-fazio, F., 1924, *Riforma med.*, **40**, 393.

Francis, E. & Evans, A. C., 1926, *Publ. Hlth. Rep.*, Wash., **41**, 1273.

Gage, E. & Gregory, D. A., 1926, *J. Amer. Med. Ass.*, **87**, 848.

Gilmour, R. T., 1906, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. IV, 3.

Gipps, A. G. P., 1891, *Trans. Epidem. Soc.* (Lond.) (1889–90), n.s., **9**, 76.

Girbal, 1923, *J. Prat.*, Paris, **37**, 7.

Gordon, M. H., 1899, *Lancet*, Lond., i, 688.

Gow, A., 1922, *S. Afr. Med. Rec.*, **20**, 475.

Harrison, H. & Wilson, G. S., 1928, *Lancet*, Lond., ii, 1338.

Hart, G. E. & Traum, J., 1925, *Tech. Pap. Calif. Agric. Expt. Sta.*, No. **19.**

Hayes, F. M. & Barger, E. H., 1922, *Rep. Calif. Coll. Agric.*, p. 238.

Holt, R. L. & Reynolds, F. H. F., 1925, *Milit. Surg.*, **56**, 414.

Holth, H., 1909, *Berl. tierärztl. Wschr.*, **25**, 686.

Holtum, A. W., 1928, *J. Comp. Path.*, **41**, 25.

Horrocks, W. H., 1905^{1}, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. I, 5 ; 1905^{2}, *ibid.*, Pt. I, 14 ; 1906, *ibid.*, Pt. IV, 27.

Horrocks, W. H. & Kennedy, J. C., 1905, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. IV, 70.

Huddleson, I. F., 1922, *Tech. Bull. Agric. Expt. Sta.*, **55**, 1 ; 1924, *ibid.*, **65** ; 1926, *J. Amer. Med. Ass.*, **86**, 943.

Huddleson, I. F. & Abell, E., 1928, *J. Infect. Dis.*, **43**, 81.

Huddleson, I. F., Hasley, D. E. & Torrey, J. P., 1927, *J. Infect. Dis.*, **40**, 352.

Huddleson, I. F., Torrey, J. P. & Carlson, E. R., 1928, *J. Amer. Vet. Med. Ass.*, **72**, 889.

Huddleson, I. F. & Winter, O. B., 1927, *J. Infect. Dis.*, **40**, 476.

Hughes, L., 1892, *Lancet*, Lond., ii, 1265 ; 1893, *Ann. Inst. Pasteur*, **7**, 628 ; 1896, *Lancet*, Lond., ii, 239 and 518 ; 1897, *Mediterranean, Malta or Undulant Fever*, London (Macmillan & Co.).

Hull, T. G. & Black, L. A., 1927, *J. Amer. Med. Ass.*, **88**, 463.

Izar, G., 1926, *Riforma med.*, **42**, 433.

Izar, G. & Mastrojeni, G., 1927, *Riforma med.*, **43**, 100.

Jaffé, R. H., 1922, *Virchows Arch.*, **238**, 119.

Keefer, C. S., 1924, *Johns Hopk. Hosp. Bull.*, **35**, 6.

Kennedy, J. C., 1905, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. III, 56 ; 1914, *R.A.M.C. Jl.*, **22**, 9.

Khaled, Z., 1921, *J. Hyg.*, Camb., **20**, 319 ; 1924, *ibid.*, **22**, 335.

Kolesnikoff, H., 1925, *Trav. Inst. Microbiol. Hyg. Azerbaidjan*, **2**, 139.

Kolle & Hetsch, 1911, Quoted by Mohler & Eichhorn in *Rep. U.S. Bur. Anim. Ind.*, No. 28, p. 119.

Kristensen, M., 1928, *Zbl. Bakt.*, Abt. I, Orig., **108**, 89.

Larsen, W. P. & Sedgwick, J. P., 1913, *7th Ann. Rep. Amer. Ass. of Med. Milk Commission*, 1911.

Little, R. B. & Orcutt, M. L., 1923, *J. Exp. Med.*, **35**, 161.

Lubbehusen, R. E., Fitch, C. P. & Boyd, W. L., 1926, *Cornell. Vet.*, **16**, 166.

McAlpine, J. G. & Slanetz, C. A., 1928, *J. Infect. Dis.*, **43**, 232.

McFadyean, J., Sheather, A. L. & Minett, F. C., 1913, *J. Comp. Path.*, **26**, 142.

McFadyean, J. & Stockman, S., 1909, *Report of Departmental Committee on Epizootic Abortion*, London, Appendix to Pt. I, 4.

Manson-Bahr, P. H., 1927^{1}, *Lancet*, Lond., ii, 1414 ; 1927^{2}, *Abh. Ausldsk. Hamburg Univ.*, **26**, 274.

Mathews, F. P., 1926, *J. Amer. Vet. Med. Ass.*, **69**, 613.

Mediterranean Fever Commission Report, 1905–7, *Reports of Commission appointed by Admiralty, War Office and Civil Govt. of Malta, under the Supervision of Advisory Comm. of Roy. Soc., London* (Harrison & Sons), 7 parts, Bibliography in Part V.

Meyer, K. F., Fleischner, E. C. & Shaw, E. B., 1919, *Proc. Soc. Exp. Biol.*, N.Y., **16**, 152 ; 1922, *J. Infect. Dis.*, **31**, 159.

Meyer, K. F. & Shaw, E. B., 1920, *J. Infect. Dis.*, **27**, 173.

MOHLER, J. R. & EICHHORN, A., 1911, *Rep. U.S. Bur. Anim. Ind.*, No. 28, p. 119.
MOHLER, J. R. & TRAUM, J., 1911, *Rep. U.S. Bur. Anim. Ind.*, No. 28, p. 147.
NÈGRE, L. & RAYNAUD, M., 1911, *C.R. Soc. Biol.*, Paris, **70**, 472 ; 1912, *ibid.*, **72**, 791 and 1052.
NICOLLE, C., 1905, *C.R. Soc. Biol.*, Paris, **59**, 242.
NICOLLE, C. & CONSEIL, E., 1909, *C.R. Soc. Biol.*, Paris, **66**, 503.
NICOLLE, C., BURNET, ET. & CONSEIL, E., 1923, *C.R. Acad. Sci.*, Paris, **176**, 1034.
NICOLLE, C. & PRATT, J. S., 1915, *Amer. J. Dis. Child.*, **10**, 203.
NOWAK, J., 1908, *Ann. Inst. Pasteur*, **22**, 541.
ODDO, C., 1925, *Bull. Inst. Pasteur*, **23**, 1091.
ORPEN, L. J. J., 1924, *Trans. Soc. Trop. Med. Hyg.*, Lond., **17**, 521.
PONTICACCIA, L., 1925, *Giorn. Clin. med.*, **6**, 218.
PREISZ, H., 1903, *Zbl. Bakt.*, Abt. I, Orig., **33**, 190.
QUINLAN, J., 1923, *Arch. wiss. prakt. Thierhlk.*, **49**, 192.
REICHEL, J. & HARKINS, M. J., 1917, *J. Amer. Vet. Med. Ass.*, **50**, 847.
ROY. COMM. REP., 1907, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. VI, p. 70.
RUNNELLS, R. A. & HUDDLESON, I. F., 1925, *Cornell. Vet.*, **15**, 376.
SCHROEDER, E. C., 1922, *J. Amer. Vet. Med. Ass.*, **60**, 542.
SCHROEDER, E. C. & COTTON, W. E., 1911, *Rep. U.S. Bur. Anim. Ind.*, **28**, 139 ; 1916, *J. Amer. Vet. Med. Ass.*, **50**, 321 ; 1924, *ibid.*, **64**, 479 ; 1925, *ibid.*, **66**, 550.
SCOTT, R. W. & SAPHIR, O., 1928, *Amer. J. Med. Sci.*, **175**, 66.
SEDALLIAN, J. & P., 1924, *C.R. Soc. Biol.*, Paris, **90**, 1435.
SEDDON, H. R., 1919, *J. Comp. Path.*, **32**, 1.
SEDGWICK, J. P. & LARSEN, W. P., 1913, *Amer. J. Dis. Child.*, **6**, 326 ; 1915, *ibid.*, **10**, 197.
SENSENICH, R. L. & GIORDANO, A. S., 1928, *J. Amer. Med. Ass.*, **90**, 1782.
SERGENT, E., GILLOT, V. & LEMAIRE, G., 1908, *Ann. Inst. Pasteur*, **22**, 209.
SHAW, E. A., 1905[1], *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. I, 95 ; 1905[2], *ibid.*, Pt. III, 56 ; 1906, *ibid.*, Pt. IV, 8.
SMITH, THEOBALD, 1919, *J. Exp. Med.*, **29**, 451 ; 1924, *ibid.*, **40**, 219 ; 1925, *ibid.*, **41**, 639 ; 1926[1], *ibid.*, **43**, 317 ; 1926[2], *ibid.*, **43**, 207.
SMITH, THEOBALD & LITTLE, R. B., 1923, *Monog. Rockefeller Inst. Med. Res.*, No. 19.
SOULEYRE, C., 1921, *Bull. Soc. méd. Hôp. Paris*, **45**, 176.
STAFSETH, H., 1920, *Tech. Bull. Mich. Agric. Expt. Sta.*, **49**, Pt. II.
TAPIA, M. & DE NICHOLAS, M., 1926, *C.R. Soc. Biol.*, Paris, **95**, 563.
THOMPSON, T., 1928, *Lancet*, Lond., ii, 1335.
TINTI, M., 1925, *Riv. clin. Med.*, **26**, 649.
TOD, A. T., 1929, *Lancet*, Lond., i, 388.
TRENTI, E., 1923, *Policlinico* (sez. prat.), **30**, 1249 ; 1925, *ibid.*, (sez. prat.), **32**, 767.
VERCELLANA, G. & ZANZUCCHI, A., 1926, *Pathologica*, **18**, 247 and 395.
VINCENT, H., 1918, *C.R. Acad. Sci.*, Paris, **166**, 359 ; 1922, *Bull. Acad. Med.*, Paris, **87**, 672.
WALL, SVEN, 1914, *Rep. 10th Internat. Vet. Congr.* (London), **2**, 292.
WILSON, G. S. & NUTT, M. M., 1926, *J. Path. Bact.*, **29**, 141.
WORDLEY, E., 1927, *Lancet*, Lond., ii, 1290.
WRIGHT, A. E. & SMITH, F., 1897, *Lancet*, Lond., i, 656.
ZAMMIT, T., 1907, *Rep. Mediterranean Fever Commission to Royal Society*, London, Pt. VI ; 1922, *Ann. Trop. Med. Parasit.*, **16**, 1.
ZIEMANN, H., 1921, *Deuts. med. Wschr.*, **47**, 500.
ZWICK & KRAGE, 1913, *Berl. tierärztl. Wschr.*, **29**, 41.

CHAPTER X. *BACILLUS ANTHRACIS.*

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History and Occurrence.

ANTHRAX (*French* charbon, maladie charbonneuse; *German* Milzbrand) is a disease which attacks many animals and is transmissible from them to man, and occurs in most parts of the world. The name is derived from the Latin *Anthrax*, a carbuncle, which comes from the Greek ἄνθραξ, a live coal or carbuncle. The disease is caused by a well-defined bacillus which historically is of interest as being the first micro-organism definitely to be associated with a disease. This bacillus was discovered by Rayer in 1850, and was afterwards studied in detail by Koch (1877) and Pasteur (1880) (for the history of Anthrax see Vol. I). Human anthrax seems hardly to have been recognized in Great Britain until the publication of W. Budd's description of it in 1863.

Anthrax is of importance economically on account of its occasionally devastating incidence among flocks and herds, less so in Great Britain, where in 1926 only 845 cases in animals were reported, than in some European countries such as Italy, Hungary and Russia; in the last named more than 43,000 animals died of the disease in the first ten months of 1914. In the East it must also be very prevalent, judging by the frequency with which raw materials, such as hides, bristles, wool and hair, imported into Europe are found to be infected, but the reported figures are unreliable. Until more precise information as to the prevalence of anthrax in India, South Africa, &c., is available no effective steps can be taken for dealing with outbreaks there, nor for minimizing by local measures the risks attendant upon the handling of the products of these countries' herds.

Human anthrax is infrequent in this country, and appears to be diminishing; thus, in 1913, 101 cases of anthrax in man were reported, in 1923 there were 46 cases, and in 1927, 38 cases with 3 deaths. Though this diminution may be the result of the precautionary measures now taken, it must be remembered that less raw material has been handled during the last few years owing to trade depression in the industries concerned.

Anthrax in man is closely linked with industries and occupations in which he is brought into contact with certain animals, such as cattle, sheep, goats, pigs and elephants, and their products. These products are mostly the raw materials, but the finished article, such as shaving-brushes, blankets and cardigan jackets, and even sausages, may convey the infection. Synonyms for the disease thus arise, derived from occupations (e.g. wool-sorters' disease, Hadernkrankheit [rag-disease]), or from the locality in which an industry is carried on (e.g. maladie de Bradford).

Characters of Bacillus anthracis.

B. anthracis is related to several organisms which occur in the soil and in dust. They are all characterized by considerable size, a preference for aerobic conditions for growth, active spore-formation, by their affinity for basic aniline dyes and positive staining by Gram's method, and by their modest demands upon culture media. The forms other than *B. anthracis* are, however, generally motile and avirulent (vide Chapter VIII). In the process of evolution it is conceivable that *B. anthracis* was derived from one of these saprophytic organisms, but it is questionable if it is ever met with now except in connection with the infected animal or with products derived therefrom, though if implanted in the soil it may maintain its vitality for long periods, and certain pastures and districts are notoriously infected.

MORPHOLOGY.

B. anthracis occurs in the blood and organs of infected animals as a straight rod measuring 5 to 10μ long by 1 to 1·5μ broad, sometimes single, sometimes united to form short chains of two, three or a few bacilli ; the number varies somewhat in different animals. In the unstained condition the united bacilli of a chain appear to form a homogeneous filament, but on staining, the constituent bacilli are seen to be separated from one another by a small though definite interval. The young unstained bacillus is homogeneous and practically structureless, but when older may be finely granular. It is always non-motile—an important distinction from some of its saprophytic relations. Motility, it is true, has been claimed by some observers, but the claim cannot be admitted as being correct. Thus Dupond (1905) described a slight waving movement of the end of a filament, but as the observation was made in a " wet " specimen examined with a water-immersion lens without a cover-glass, the action of currents in the fluid cannot in such circumstances be excluded.

Spores never develop in the bacilli within the infected animal ; free oxygen is necessary for their development.

B. anthracis is a capsulated organism. This capsule, first demonstrated by Johne (1892–3) and Lüpke (1895) was at first thought to be characteristic only of the bacillus in the blood and tissues, being more difficult to demonstrate in cultures. Hence some have claimed it to be an artefact, but it is now generally recognized to be an integral part of the organism. While Kern (1897) believed that each bacillus has it own capsule, Johne stated that a small chain of bacilli may be enclosed within one capsule. The latter is true for the bacilli present in the tissues, while a capsule to each bacillus is the rule in cultures. The capsule may lie closely apposed to the bacillus or may be raised, bulla-like, in which event the bacillus itself is generally irregularly shaped, granular, and disintegrating. The capsules are sometimes empty, especially in old cultures. Capsules of unusual size may develop around the organism grown in some culture media, e.g. brain-agar at 42 to 43° C. (Podvisotski and Taranukhin, 1898). The capsule presumably serves as a protective mechanism. Hess (1920), for instance, is of opinion that the capsule protects the bacillus against phagocytosis, and holds that only the encapsulated organism is virulent. Morihana (1921) examined strains of anthrax bacilli obtained by Bail seven years previously, and deprived by him of much of their virulence by cultivation at high temperatures, and found that they were non-virulent and had also lost the power of capsule formation, with the exception of one strain which had regained virulence and, at the same time, capsule formation.

Under artificial cultivation, and especially in broth cultures, the chains of *B. anthracis*, short in the blood and tissues, grow out into long and sinuous filaments, their length limited only by the confines of the medium. These filaments, which never branch, are composed of bacilli placed end to end, with a small interval between each, just as in the chains in the tissues. The constituent bacilli of these filaments vary somewhat in size in different media and at different ages ; from a young agar culture they average 3·5 to 4·5μ in length by 0·75 to 1·0μ in breadth. As they become older they tend to lose in some degree their regular cylindrical shape, may appear shorter, and swollen involution forms may make their appearance. Haag (1927) describes the formation of gonidia in the course of development of the anthrax bacillus.

Under ordinary conditions and in the presence of free oxygen, spores always develop in cultures. In a surface agar culture at 37° C. spores begin to be formed within a few hours, and in 20 to 24 hours become fully developed, nearly every individual bacillus containing one. The spore, which was discovered by Koch, commences as a small, bright, refractile point, which increases in size and gradually grows into an ovoid body situated at or near the centre of the bacillus. The long axis of the spore lies in the long axis of the bacillus, and the short diameter of the spore is a trifle less than that of the rod in which it has been formed, so that there is little or no bulging of the rod. During the development of the spore the protoplasmic contents of the bacillus become granular, and the spore has been stated to be formed by the collection and coalescence of certain chromatin-like granules present in the cytoplasm. With the full development of the spore, the rod in which it has been formed gradually breaks up and disintegrates, liberating the spore, which still, however, may carry a tag of bacillary protoplasm for some time. In a culture a few days old, little else than spores may be visible, the bacilli having almost completely disintegrated and disappeared.

In the culture which has spored, the spores may lie dormant and unaltered for long periods, but if the spore be transferred to a fresh medium and incubated, it germinates and grows into the original vegetative bacillary form. In doing so, the spore increases somewhat in size, its contents become less refractile, the containing membrane ruptures at one point, from which a protoplasmic bud appears and grows into the bacillus. The bacillus thus formed may sometimes be seen enveloped at one end with the remains of the spore membrane.

Prazmowski (1885) stated that when the spore germinates, the bud appears at one end of the long axis, i.e. germination is polar, but others believe that germination is both polar and equatorial.

The spore consists of cytoplasm contained within a definite membrane which according to some consists of two layers. The spore cytoplasm probably contains more protein and less water than the bacillary cytoplasm, and this may be one of the factors upon which the resistance of the spore to heat depends.

The power of spore formation of *B. anthracis* is influenced by many conditions and may by artificial means be more or less permanently suppressed ; this will be dealt with later (p. 445).

Variations from the ' type '. One of the most important variations is the artificial production of asporogenous races which breed true. Involution forms occur in cultures some days old. From cultures in gelatin and glycerin agar very striking preparations are sometimes obtained with numerous large spherical and lemon-shaped elements ; these have long been known and have been fully described by Lutz (1922). Involution forms are often found in asporogenous cultures in whatever way the loss of spore-formation has been brought about (Abt, 1921). Ducloux (1924) observed variation by growing the organism in a fish-liver broth ; the bacilli may become bent or hook-shaped, with swollen ends, or the ends may be rounded and the bodies of the bacilli indented laterally.

More profound alterations have been described by several observers. It is to be noted that *B. anthracis* as ordinarily met with is one of the most constant of bacterial species in all its characters, and a survey of the papers raises considerable doubt how far these observations are correct. Little information is usually given of any adequate controls, and when the mutations are claimed to have arisen in inoculated animals, the possibility of contamination is ever present. Thus, Chauveau and Phisalix (1895) isolated a terminal-spored, Gram-negative, non-pathogenic bacillus from animals inoculated with attenuated cultures (named *B. anthracis claviformis*). A reference to the original paper and the illustration accompanying it shows that the supposed variety was a contaminating saprophyte. Zironi (1919) and also Sanfelice (1924) describe Gram-negative cocco-bacillary forms, obtained by the former from an inoculated rat (named *B. anthracis colisimile*), and by the latter by cultivation in an oxygen-free medium. V. Henri (1914) described extraordinary mutations resulting from exposure of anthrax cultures to ultra-violet radiations—coccoid forms and slender filamentous forms which were Gram-negative and non-liquefying. Inoculated into guinea-pigs, death followed in from 10 to 20 days, with abscesses in the spleen and other organs—an event unknown in anthrax infection ; much more confirmation of these various observations is, therefore, necessary before they can be admitted as correct, and the subject of variation of *B. anthracis* merits further investigation.

Gratia (1924) confirms an observation of Markoff in 1912, describing two types of anthrax colonies, one flat, finely granular, with irregular edges ; the other convex, smooth, glistening and homogeneous, with regular edges. The material of the first type of colony is friable and stringy and grown in broth it yields a culture which spontaneously agglutinates and deposits in flakes. Microscopically, the growth consists of skeins of long filaments made up of long and thin bacilli. The other type of colony is creamy in consistence and in broth gives a diffuse growth. Microscopically, the growth consists of bacilli which are thicker and are isolated or form only short chains. These colonies would seem to correspond with the ' rough ' and ' smooth ' colonies that other organisms develop, but with *B. anthracis* it is the rough variety that is the more virulent. This subject needs further investigation.

Staining. The bacillus is well stained by any basic aniline dye and is Gram-positive, and the capsule can be readily demonstrated by the usual methods. But in the process of dissolution (as during putrefaction) Gram-negative forms tend to appear, and the bacillus becomes more difficult to stain. When this is the case, Foth (1919) recommends the use of Giemsa's stain. Churchman (1927) recommends the Burke modification as being the best Gram-stain for *B. anthracis*, and finds that with the Burke technique young cultures are definitely and constantly Gram-positive. He also observed a peculiar reversal of the Gram reaction. If the anthrax bacilli are treated with a very dilute solution of such dyes as acriviolet, gentian violet, or acriflavine (0·5 c.cm. of a thick suspension of a young culture with one loop of a 1 per cent. aqueous solution of the dye), and after a lapse of several hours preparations are stained by the Gram process, a considerable proportion of the bacilli will now be Gram-negative. Churchman suggests as a probable explanation of this phenomenon that *B. anthracis* consists of a Gram-positive cortex that is easily destroyed by lysis and by degeneration, and is notably sensitive to the action of certain dyes, and that when the cortex is destroyed the slender Gram-negative medulla comes into view. It seems highly probable that an action of this kind accounts for some of the supposed Gram-negative varieties of *B. anthracis* which have been described by Henri and others.

The spores of *B. anthracis* may be readily stained by the usual methods.

CULTIVATION AND CULTURAL REACTIONS.

The bacillus—which is a facultative anaerobe—makes few demands upon culture media. It grows best at 35° C. but will develop at a temperature so low as 14° C. and so high as 43° C., but spore-formation does not take place at the extremes of this scale. The reaction of the medium should be neutral or slightly alkaline ; the optimum for broth is pH 7·8 to 8·1, for agar pH 7·5 to 7·8. In broth a flocculent turbidity develops, and as the flocculi become more dense they fall to the bottom of the tube leaving the supernatant broth clear. Gelatin is liquefied. When grown on the surface of gelatin a greyish-white colony appears about the second day, becoming granular as it increases in size, with rounded wavy edges composed of straying loops of filaments tending to turn back to the main mass of the colony, the general aspect of the colony being likened to a barrister's wig. In older colonies the filaments tend to break up so that their characteristic arrangement is more or less lost, and the colony becomes flocculent and gradually sinks in the trough of liquefied gelatin. In gelatin stab-cultures (preferably 5 to 7½ per cent. gelatin) the border filaments are more conspicuous, growing into the gelatin at right angles to the line of the stab, and longer at the top, thus giving the culture the appearance of an inverted fir tree. As the liquefaction of the gelatin proceeds from top to bottom these appearances are gradually lost, a flocculent deposit being the final remnant.

On agar the growth forms a whitish-grey pellicle the edges of which are similar to those seen on gelatin plates, but whereas isolated colonies on gelatin are usually spherical those on agar tend to take the shape of a scalene triangle with more or less rounded angles. If a recent colony of this kind is touched with a needle it can be pulled off the medium as a thread ; if not too large, the whole colony can be lifted. As the colony grows older it becomes granular and its constitutent filaments become less distinct and gradually break up. If the colony is large enough—and especially in streak-cultures—an appearance develops after 24 hours as of numerous air bubbles behind a gauzy film or behind frosted glass, which can be recognized in cultures many months old. This is not met with in cultures of organisms which otherwise resemble anthrax cultures closely, although colonies composed of curling filaments are formed by a number of other spore-bearing bacilli. Where such similarity exists, Eurich (1912) has found deep agar cultures to offer more distinctive features. Tubes of melted peptone agar are inoculated and poured into Petri dishes, 9 to 10 c.cm. being used for dishes 3 to 4 inches in diameter. ' The resulting colonies are of a clear greenish grey when examined under × 50 magnification and resemble coils of fine twine into which knot upon knot has been tied. From the main mass of such a colony a few tendril-like outgrowths pass into the surrounding agar—not radiating from a centre but springing from any part of the colony. Should one of these tendrils reach the surface of the agar, a delicate surface growth will then appear. At times some short delicate hairs, rarely spicules, beset the tendrils, but they are never very numerous and may be absent '.

The anthrax bacillus has but a feeble hæmolytic action on blood-agar or in blood-broth. In plate cultures on blood-agar there is a transparent zone in the immediate vicinity of the colony only, whereas anthracoid and other non-pathogenic spore-bearing organisms not only hæmolyse more rapidly but give rise to a broad, sharply defined transparent halo around their colonies. These differences are best seen on 5 per cent. blood-agar after 16 to 24 hours' incubation, becoming obscured after 36 hours. But since, as Hallermann (1925) points out, the hæmolytic power of anthrax bacilli may be increased by cultivation in some media (e.g. alcohol-broth) the possibility of a similar increase being brought about under natural conditions must be borne in mind. The addition of bile to the medium does not seem to prevent the growth of the anthrax bacillus nor to affect its virulence.

The growth on potato is not characteristic—a whitish film which increases rapidly in density and becomes yellowish with age, and, later, brown.

In fluid blood-serum the anthrax bacillus behaves as it does in broth. Milk is slowly coagulated, the clot being subsequently digested, and the reaction either remains alkaline or becomes faintly acid. On coagulated blood-serum a dull white pellicle forms, becoming greyish after a few days with partial liquefaction of the medium ; but if the serum has been inspissated at 70° C. the growth is feathery (Eppinger, 1894).

These are the classical forms which anthrax cultures may assume ; but some reputed varieties and mutations are described as showing a departure from type in their modes of growth also. Thus Rosenthal stated that by growing the bacillus in a succession of cultures of diminishing oxygen-content he could develop a strain that was anaerobic and no longer liquefied gelatin.

Modifications of Spore-Formation.

Pasteur found that in old gelatin cultures the power of sporulation is sometimes lost. The ability to form spores may be increased or diminished, even to abolition, by artificial means. It may be favoured—often, but not invariably—by the addition of ascitic fluid or of alkalized lemon juice to the culture medium. Cultivation below 16° C. and above 42° C. prevents sporulation, though refractile granules may form within the bacilli (the ' false spores ' of Chauveau). Bacilli that have become asporogenous under these conditions will develop spores when grown at the usual temperature, but the addition of certain substances to the culture medium may bring about a permanent loss of spore-formation. Thus, the addition of phenol in an average proportion of 1 : 800, or of bichromate of potassium in a proporiton of 1 : 2,000 (Chamberland and Roux) may have this effect. The former method is the better, but its effect on different strains varies and it may occasionally fail. Other methods are too uncertain ; thus Bormans (1896) found that the anthrax bacilli grown in the sera of the horse, dog, sheep and calf may fail to form spores and that repeated sub-culturing in these media may finally lead to permanent loss of power of sporulation. The substances in the sera that exert this inhibition are not destroyed at a temperature of 55° C. Growth on media of vegetable origin, e.g. prepared from the tissues of *Opuntia vulgaris*, may bring about the loss, for several generations, of the power to form spores (Ducloux, 1920). On glycerinated potato sporulation may be lost, only to return when cultivation on agar is reverted to.

Separation of spores from bacilli (Dold and Weyrauch, 1924) may be effected by the application of heat, of antiformin and of urea. Of these urea is the least objectionable as it affects the spores least. As the power of resistance of spores varies with the strain, spores of known high resistance should be selected for testing disinfectants. Reiter (1920) recommends Heider's wheat-extract-agar for obtaining spores of high resistance (tested against steam), though it may fail owing to causes at present unknown.

Primary Isolation.

How primary isolation is to be effected will depend (*a*) upon the material in which the presence of the organism is sought, and (*b*) upon the special difficulties likely to be encountered ; these factors are often closely linked. Isolation from the human or animal body may be difficult or doomed to failure : (1) because of the scantiness of the bacilli in the blood-stream or their unequal distribution in the tissues ; and (2) because of the disintegrating action of the blood-plasma or of the process of putrefaction upon the bacillus. If the patient or animal is still alive, or quite recently dead, and cultures are made without delay, little difficulty will generally be encountered, provided the pathologist knows what organs and tissues will best repay examination. From ' malignant pustules ' bacilli can generally be grown on peptone agar in abundance from the contents of vesicles or from the serum obtained on lightly scraping the edges of the sore. They can be grown from sputum and pleural exudates, and from the vomit, in pulmonary and gastro-intestinal anthrax respectively ; but as several c.cm. of material may need to be examined plate cultures are here the more serviceable. If cultures are to be made from blood, 10 to 20 c.cm. will be required for broth cultures or for agar plates. If the body is more than 24 hours dead, and even earlier in hot climates, the lytic changes above mentioned militate against success. It is then advisable to make cultures not only from the spleen, heart's-blood, lungs and exudates, but also from peripheral veins as remote as possible from sources of putrefaction.

In the examination of raw materials such as wool, hair, bristles, hides, bone manure, and of other products suspected of contamination, such as corn and hay and oilcake, the differentiation of the anthrax bacillus from other spore-bearing bacilli is the principal difficulty. Reichel (1911) has tried to circumvent the tendency of these bacilli to crowd out or overgrow the colonies of the anthrax bacillus by using Petri dishes 20 cms. in diameter. Laubenheimer (1911) recommends that wool and hair be cut up into short pieces, placed in a Petri dish and have agar poured upon them. Eurich (1912) has found the following method to be the most useful in the examination of wool, hair, &c. : the material (the quantity will depend upon its condition—whether clean or laden with blood, excrement or mud) is put into a flask with 50 to 100 c.cm. of sterile water, and 3 to 5 c.cm. of a 5 per cent. solution of caustic potash are added. After standing for a time, or if much blood-stained, incubating at 37° C. for some hours, the contents of the flask are poured into a flat dish, and the wool or hair is teazed out. The wash is then poured off into a fresh flask and is heated to 80° C. and kept at this temperature for some minutes. The heated wash is then plated, $\frac{1}{3}$ to $\frac{1}{2}$ c.cm. being added to at least 9 or 10 c.cm. of melted agar, and the mixture is poured into a Petri dish 4 inches in diameter. Several plates should be prepared. The more agar is used the greater will be the proportion of deep-lying colonies and the less will be the danger of surface overgrowth of dust organisms ; the deep colonies of anthrax are the more characteristic ones. When blood is not present, i.e. when the prevention of coagulation by the addition of caustic potash is not needed, a modification of Endo's fuchsine-agar is the most useful medium. Jänisch's original modification consisted in using an agar containing 10 per cent. of peptone instead of 1 per cent. and 4 per cent. of agar instead of 3 per cent. ; but Meyer (1920) has shown that 1 per cent. of peptone will suffice if 4 per cent. of agar be employed. The 10 per cent. solution of sodium sulphite for the reduction of the fuchsine should be added immediately before use ; the addition of 45 c.cm. of a 10 per cent. solution to 1 litre of agar giving the best results. By this means the growth of the anthrax bacillus is favoured, and that of other spore-bearing organisms retarded, especially *B. subtilis* and *B. mesentericus*. Enoch (Meyer, 1920) used for the examination of fish meal a method which can be applied to other products such as oil-cake ; 200 gm. of the suspected material are stirred with sufficient distilled water to form a thin porridge, which is strained through a sieve of 1 mm. mesh ; the filtrate is allowed to stand ; 200 c.cm. of supernatant fluid are then decanted, heated in a flask for 1 hour at 80° C. cooled under water, and allowed to stand till a deposit has formed. The supernatant fluid is then centrifuged at high velocity, and the sediment is added to broth and incubated for 12 hours ; from this agar plates are inoculated and incubated on the top of the incubator, not in it.

In some cases, when the culture method fails, as it may do, the inoculation of an animal—a mouse or guinea-pig—with the material may succeed, and should in all cases be employed in addition to the culture method. An organism isolated in culture and resembling *B. anthracis* should be finally tested by animal inoculation.

The Viability of Cultures.

The viability of cultures will vary with the presence or absence of spores. Asporogenous bacilli die in the course of a few weeks, the period of viability varying greatly in different strains and with the medium ; thus, on black bread anthrax bacilli have survived for 28 days, on white bread for 37 days, while in water they succumb in 3 or 4 days. Autolysis readily occurs and Lemos Monteiro (1925) found in old cultures a lytic substance capable of acting upon young cultures, and claims that the fragments resulting from lysis may develop into bacilli possessing lysis-resisting powers. Sporulating cultures may live for several years provided they are kept in contact with oxygen and in the dark ; stored in sealed tubes they may perish within a year ; and Arloing (1885) found that in cultures exposed to sunlight the spores succumb more quickly than asporogenous bacilli, his explanation of this anomaly being that sunlight has a more powerful action upon bacilli that have just issued from spores. It has also been noted that spores survive longer in natural habitats such as old tannery pits and soil than in artificial media.

BIOCHEMICAL REACTIONS.

As has been stated in the section on ' cultivation ', the anthrax bacillus coagulates milk and then digests the curd, and also liquefies gelatin and inspissated or coagulated serum.

The action of *B. anthracis* upon fermentable substances has been determined by Kristensen (1927) for a number of strains and is as follows : glucose and trehalose are fermented rapidly with acid-production, but without gas-formation ; saccharose is fermented somewhat less rapidly, as are the following in the order given—maltose, lævulose, dextrin, starch, glycerin and salicin (inconstant). The following are not fermented : erythritol, mannitol, dulcitol, sorbitol, rhamnose, l-arabinose, xylose, mannose, raffinose, lactose, galactose, inulin, inositol. The writer (Hewlett) can in the main confirm these reactions for five strains examined. Slight differences occur with different strains—for instance, one strain acted on saccharose much less vigorously than the other four—and Buzna (1925) noted differences among virulent, avirulent and asporogenous strains examined. Kristensen claims for these reactions of *B. anthracis* a value equivalent to the biochemical reactions of *B. typhosus*, but omits reference to results of control tests with anthracoid organisms. *B. anthracoides* certainly has very similar fermentation reactions, but is motile in young culture (Grierson, 1928). Kristensen recommends for these reactions a medium containing meat-extract, 1·0 per cent. of Witte's peptone, 0·5 per cent. of sodium chloride, and 1 : 40,000 brom-thymol blue, to which, after sterilization, 0·5 per cent. of the fermentable substance is added.

The anthrax bacillus possess a reducing power upon methylene blue, and according to Gozony and Kramar (1922) a direct parallelism exists between the reducing energy of a strain and its virulence. Endo's medium is scarcely reduced and neutral-red not at all.

Bacteriophage. Pellucid areas may develop in agar cultures of certain sporing bacilli, including *B. anthracis.* Darányi (1927) considers that this phenomenon is due to the action of an autolytic ferment and not to a bacteriophage.

SEROLOGICAL REACTIONS.

The serological reactions obtainable with other bacteria are given by the anthrax bacillus also ; but with the exception of Ascoli's precipitin reaction and possibly of opsonic index determinations (see p. 463), no reliable diagnostic tests have been evolved from them.

Precipitins. Ascoli's precipitin reaction depends upon the interaction of a precipitin contained in an immune serum and a precipitinogen derived from the substance of the bacterial bodies. An extract is prepared from an organ, e.g. the spleen, of the suspected case of anthrax infection and is mixed with a special anthrax immune serum. In a positive case, a precipitate forms in the mixture.

This anthrax precipitinogen differs from the ordinary bacterial antigens in being resistant to heat, boiling and the application of even higher temperatures being well borne; it also resists preservatives, such as alcohol, as well as putrefaction. The anthrax precipitin reaction is, like all others, not absolutely specific but is a group reaction, being applicable to pseudo-anthrax bacilli also; indeed it is possible to obtain anthrax-precipitating sera containing neither protective nor curative properties by employing cultures of pseudo-anthrax strains. The practical application of the test will be dealt with later (p. 463).

Agglutination can be carried out only with a homogeneous suspension of organisms such as can be obtained with attenuated cultures of anthrax bacilli. But just as in the case of precipitins there is no correspondence between the immunizing power of a serum and its wealth in agglutinins. The normal serum of many animals such as the rat, rabbit, guinea-pig, horse, &c., will agglutinate in a dilution of 1 : 10 to 1 : 50, and normal human serum in even greater dilutions (up to 1 : 500); and as some normal sera will agglutinate as energetically as will the corresponding immune sera, agglutination as a diagnostic test is at present valueless.

The bactericidal activities of sera are also not proportional to their immunizing powers; thus, while rats and dogs yield sera that are highly bactericidal, fowls and frogs, though possessing a natural immunity, yield sera of a low standard. This lack of parallelism receives illustration in another way (Zehetmayr, 1922): a subcutaneous injection of normal ox-serum will exercise a protective influence against an injection of anthrax bacilli made into the same site, but no protection is conferred against bacilli introduced into a distant part unless very large doses of the serum are given; on the other hand, immune sera readily protect though their bactericidal power be no greater. Behring long ago demonstrated the protective action of the bactericidal serum of white rats when injected simultaneously with anthrax bacilli into mice, while Metchnikoff and Roux (1891) showed that after their separate injection at different sites the mice died, and that many white rats are susceptible to anthrax even though their sera are anthracocidal. On the other hand, Marchoux (1895) found that the sera of rabbits and sheep which had been successfully immunized were devoid of bactericidal powers *in vitro* even though they had protective and therapeutic value. Some semblance of parallelism is, however, given by the sera of young animals, which are less immune than the adult; for example, Keller states that the serum of the healthy human infant contains no anthracocidal body in the second week after birth, but that this appears in the seventh to ninth week of life; the rate of diminution of the red corpuscles and of the increase of globulin in the serum is analogous; a causal nexus has, however, not yet been determined.

Serological races such as exist among pneumococci and meningococci have not yet been identified.

Pathogenic Action.

ANTHRAX IN ANIMALS. SPECIES SUSCEPTIBILITY.

It will appear that no kind of animal enjoys absolute immunity from anthrax, though certain families, genera and species possess a high power of resistance. The carnivora are among the latter, but cases occur from time to time in the dog, cat and fox, and infection in most of the large carnivors in Zoos has been recorded (from feeding with infected meat); also in the bear. Birds, reptiles and fish are insusceptible under ordinary conditions, and insects appear to be immune, but may carry the infection, e.g. flies from carrion and from infected pelts. Other animals are more susceptible, in particular the hoofed forms and the smaller rodents such as the rabbit, guinea-pig and mouse and rat. The disease has its greatest incidence in the ox, sheep, horse and goat; the pig is frequently attacked; the camel and deer are susceptible; in the East the elephant contracts the disease and an outbreak has occurred among elephants in the London Zoo. Different races of animals vary, however, in their susceptibility; thus the adult white rat is less susceptible than the grey rat, the Algerian sheep than the British and Continental races, while British and American swine are more susceptible than Hungarian pigs. Certain animals in an otherwise susceptible herd may also show a relative insusceptibility; this has been supposed to indicate the occurrence of individual immunity. But it is conceivable that the relative immunity of individuals met with in a herd may be the result of immunization through natural infection with an attenuated virus, instances of which were observed by Pasteur, and not due to individual insusceptibility. The virulence of strains unquestionably varies, though this may be difficult to demonstrate by the inoculation of laboratory animals owing to their high susceptibility.

ACCESSORY FACTORS WHICH MAY INFLUENCE INFECTION.

Dose of Organisms. Reference is made elsewhere (p. 456) to the susceptibility of adult white rats to massive doses of culture, and Martos (1927) has determined the effect of inoculating mice with a known number of anthrax bacilli. The bacilli were obtained from the spleen of an inoculated mouse by a micro-pipette method. Table I shows the results obtained:

TABLE I.

No. of mice in series	*No. of bacilli injected into each mouse*	*No. of animals which died*	*Percentage which died*	*Average duration of life*
10	20	10	100	2·6 days.
11	10	8	73	4·3 „
20	5	13	65	4·0 „
20	2	10	50	5·2 „
50	1	14	28	5·6 „

This experiment shows how few organisms in the case of anthrax are required to cause a fatal infection, and that when the number of organisms is quite small—ten or less—a certain proportion of the animals may survive.

Site of Inoculation.—An animal may seem immune because infection occurs only if the virus enters along certain channels. Thus, pigeons, generally considered to be immune, can be readily infected by inoculation into the anterior chamber of the eye.

Besredka (1921) advanced the hypothesis that for anthrax infection to take place it is necessary for the skin to be infected. In all ordinary inoculations this, of course, happens, but by avoiding skin infection, Besredka was able to inoculate guinea-pigs subcutaneously, intraperitoneally and intravenously without infection following. Besredka came to the conclusion, therefore, that the anthrax bacillus is innocuous unless it can infect the cutaneous receptor cells, and that immunization is effective if the cutaneous receptors are immunized, as can be done by cutaneous vaccination with an attenuated culture (see p. 468). His results have been partially, but not entirely, confirmed. Plotz (1924) applied Besredka's method in another way to rabbits. He introduced virulent anthrax culture in thin glass capsules beneath the skin, sutured the small incisions, and broke the capsules at varying periods after the operation, so as to liberate the anthrax bacilli. Plotz found that most of the animals remained uninfected though they had received considerable doses of bacilli into the subcutaneous tissues. The possibility of infection diminished *gradatim* with the interval after operation, i.e. with the progress of healing of the skin incision. The rabbits that escaped infection were, however, not immune to cutaneous inoculation with a much smaller number of bacilli. So far, Plotz's experiments support Besredka's hypothesis, but it is to be noted that anthrax bacilli enclosed within glass capsules tend progressively to lose virulence and to die out.

Panton and Benians (1925) repeated in the rabbit some of the experiments of Besredka and of Plotz. They found that rabbits are readily inoculated by cutaneous scarification and die of generalized anthrax infection, but that they generally survive large intravenous doses of virulent culture given by transcutaneous inoculation through a cannula. To test the experiments of Plotz, they examined the contents of capsules that had been introduced into rabbits, and found that after some days anthrax bacilli had become very scanty, both microscopically and culturally. They conclude that the receptivities of the skin and the subcutaneous tissues of the rabbit for anthrax infection are different, and that a dose fatal in the skin may be tolerated under the skin.

Balteano (1922) repeated Besredka's experiments, and extended them by introducing sealed tubes containing anthrax culture into the peritoneal cavity and afterwards breaking them ; he concludes that in the rabbit and guinea-pig the skin is the only organ susceptible to anthrax infection.

On the other hand, Bachmann, Beltrami, and Romat (1923), Combiesco (1923), Gratia (1924), and Uschida (1926), all obtained fatal anthrax infection in rabbits, guinea-pigs or mice by subcutaneous, intravenous or intraperitoneal inoculation, the greatest care being taken to avoid infecting the skin. Katzu (1925) tested Besredka's two assertions : (*a*) that infection of laboratory animals is easy by the skin, but unsuccessful by other routes if infection of the skin be avoided ; and (*b*) that this special relation of the bacillus to the skin enables immunization to be more readily effected by this path. Though he confirmed the great vulnerability of the skin, he found that the possibility of infection by other channels must be conceded. Wollman (1925) likewise succeeded occasionally in producing a fatal infection intraperitoneally to the definite exclusion of accidental cutaneous infection.

Various explanations have been given to account for these divergencies. Individual peculiarities in the animals rather than the method employed may account in some instances for success or failure ; or Besredka's more favourable results may have been due to attenuation of his laboratory stock-cultures—the result of long-continued growth on artificial media. Gratia considers that there are two strains of *B. anthracis* characterized by differences in the colonies and growths (see p. 443), and that one is much more virulent than the other. Combiesco suggests that the result obtained by different routes of inoculation depends partly upon the dose of anthrax administered. If the dose does not exceed a certain amount, the bacilli in an intravenous injection are immediately disposed of by phagocytosis, but if the dose is larger some bacilli escape phagocytosis and a fatal infection then ensues. The development of capsules by the bacilli is adduced as another explanation ; thus Gratia urges that this easier infection by way of the skin is dependent not upon the hypothetical existence of receptor cells in it, but solely upon conditions more favourable to the development of the bacillus ; in the skin the bacilli have more time, in the absence of phagocytes, to become capsulated and thus to become more resistive, but if leucocytosis is produced in the skin by infiltration with broth, its susceptibility to anthrax can be diminished. But capsule-formation alone does not suffice ; thus, one guinea-pig tolerated an intraperitoneal dose of capsulated bacilli twenty-five times larger than that which was fatal to another. Wollman (1925), therefore, advances another explanation in place of Gratia's. He argues that as accidental infection of the skin with less than the fatal dose of bacilli seems to favour infection after intraperitoneal inoculation, the fatal result must be due to a conjoint action of germs within and without the skin ; and having obtained a fatal result in 7 out of 8 guinea-pigs to which he had, in addition, given cutaneously an injection of defibrinated and sterilized fluid obtained from cutaneous anthrax œdema, while controls gave a converse result, he concludes that aggressins are formed in special abundance in the skin and that these aggressins can induce immunity, as shown by Bail. Combiesco (1924) argues that in cutaneous vaccination the bacilli accommodate themselves to the organism before they enter the circulation ; if this accommodation be artificially induced, e.g. by cultivation in oxalated blood, immunity can be produced by the subcutaneous route. This would, however, still leave unexplained Besredka's claim to have produced cuti-immunity by means of dead bacilli.

With regard to other routes of infection in anthrax, Sanarelli (1925) found that large numbers of anthrax bacilli or of spores may be given by the mouth to laboratory animals without infecting, and that the blood of an infected animal may be injected *per anum* without harm. Virulent anthrax spores enclosed in small gelatin capsules may be swallowed by mice and guinea-pigs without harm, though virulent spores can be recovered from the fæces for a week (Holman, 1922–3). These results contrast strangely with the frequency of alimentary anthrax in horses ; it is possible that sharp fragments of straw and other vegetable tissues cause slight wounds of the intestinal mucosa in the last named and thus facilitate infection. That slight wounds may produce this effect is shown in the case of a fox which met its death from anthrax through having swallowed a sharp piece of bone from the pickled head of an anthrax-infected pig. Similar lesions may account for Sobernheim's success in infecting guinea-pigs via the stomach. Experiments by Hruska (1928) also suggest that trauma exercises an influence in the development of alimentary anthrax infection. Kids were taken from the mother as soon as born and bottle-fed, so as to avoid damage to the intestinal mucosa by fodder, and when a fortnight old ingested anthrax spores without harm. Other animals similarly reared were wounded in the neck and were then given the anthrax spores by means of a stomach tube or *per rectum* and some of these animals, though not all, succumbed to anthrax infection.

Sanarelli (1924) found the digestive juices strongly bactericidal both *in vitro* and *in vivo*, failing to infect the bowel via the rectum or through a stoma in the appendix. In sterile intestinal secretions the anthrax bacillus died in 24 hours, and the development of bacteria from spores was inhibited. The gastric juice of young animals killed spore-free bacilli in 2 minutes. This bactericidal power of the gastro-intestinal digestive juices may be lost in disease though the inhibitive action upon the spores may be retained. Uschida's (1926) results with percutaneous and oral administration varied greatly, due, he thinks, to individual variations in the resistance of the animals. When food rich in anthrax spores was fed to white mice, the organism could in half the animals be recovered from the glands.

The respiratory mucous membrane of the rabbit is similarly resistant to infection. Sanarelli (1925) found that a considerable number of anthrax spores (up to 50,000) may be introduced into the lung of a rabbit without harm, but if the dose is larger, 100,000 or more, phagocytosis is insufficient and a fatal septicæmia ensues. The spores introduced may, however, lie latent in various tissues and organs, and be released therefrom and produce fatal results if the foci are distributed by various agencies—chemical, thermal, nutritional, &c.—and the rabbit which has survived a moderate dose of spores may later succumb to anthrax if kept on a very dry diet.

A similar obstacle to the entrance of anthrax bacilli and their spores is offered by the conjunctiva (Aitoff, 1922) except, so it would appear, in the case of animals with young; nor will the bacilli enter through conjunctival ulcers.

Mixed infections. We possess no information of the influence of another infection upon anthrax infection naturally acquired, but experimentally it has been shown that some other organisms antagonize an anthrax infection, notably *B. pyocyaneus*. Bouchard (1889) first showed that if guinea-pigs and rabbits were inoculated with anthrax culture or blood, and some hours afterwards *B. pyocyaneus* culture was injected, the animals were saved, but he gives no details. This work was much extended by Emmerich and Löw (1899). They confirmed Bouchard's observation that an injection of *B. pyocyaneus*, simultaneously with, or shortly after, inoculation of *B. anthracis* would save the animals. They ascribed this result to the action of an enzyme ' pyocyanase ' formed by *B. pyocyaneus*, which they extracted from its cultures (see Vol. IV). They found that pyocyanase would dissolve anthrax bacilli *in vitro*, and that *in vivo* it prevented and cured anthrax infection, just as the culture did. Further, rabbits could be immunized by several intravenous and subcutaneous injections of pyocyanase against a subsequent inoculation with virulent *B. anthracis*. Frank (1899) observed that some organisms, such as staphylococci, can inhibit the action of the anthrax bacillus. He found that if guinea-pigs were injected with staphylococci a few minutes after an injection of anthrax bacilli at the same spot, death was delayed or prevented. Their simultaneous injection gave rise to suppuration only. Even better results were obtained when mice were used; but diminished virulence of the spores and variation in the susceptibility of the animals used (a variability specially noticeable in mice) were probably not sufficiently taken into account by him. Silberschmidt and Schoch (1920) state that Friedländer's bacillus, *B. typhosus*, *B. coli* and *B. pyocyaneus*, if injected simultaneously with anthrax bacilli, prove antagonistic in guinea-pigs and mice. No antagonism is evinced if the injection of these organisms is delayed for 8 hours or if they are dead; nor is immunity acquired by the surviving animals. That *B. coli* is antagonistic to anthrax bacilli is also claimed by Sanarelli and by Gundel (1927). An interesting observation has been made by Fürst (1920): namely that there is a definite, though not absolute, antagonism in rabbits between cow-pox vaccine and anthrax. He suggests that if it can be demonstrated in larger animals also, this antagonism might be utilized in the immunization of cattle with anthrax vaccine in order to prevent the losses (by death) incurred in the process.

Other factors influencing infection. Natural resistance to anthrax infection may be artificially reduced in many ways. London's experiments on decerebrate pigeons are well known, and alcoholic poisoning, intravenous injection of powdered charcoal, and splenectomy all lower resistance. Chilled chickens and warmed frogs may be infected, and Buchner showed that fatigue lowers the resistance of adult white rats, which are relatively insusceptible. Much has been made of these factors, but in the end they prove little more than that any serious interference with the health of the animal, like the procedures named, or actual disease, will lower a natural insusceptibility to anthrax infection. Young animals are almost always more susceptible than adults.

Course of the Disease in Animals.

Natural anthrax infection in animals generally runs a very rapid course, and death often ensues without illness being noticed or even suspected.

Natural anthrax infection in the horse, ox, sheep, pig and goat is generally alimentary in origin, and is not transmitted from beast to beast by contagion. In the horse and ox local cutaneous infection occasionally occurs.

In the horse the disease may assume two forms ; one, the commoner, a malignant form, is septicæmic in character, and usually runs its course rapidly within from 8 to 30 hours, without external manifestations ; the other, from which recovery is possible, is milder and characterized by massive swellings about the head, neck and shoulder. In the ox the disease is commonly septicæmic, and may be either fulminating, with death in 2 or 3 hours ; acute (the most frequent form), with death in from 10 to 24 hours ; or subacute, with death in from 2 to 5 days ; from the subacute form there is some prospect of recovery. An external form, due to cutaneous infection, also occasionally occurs, in which large swellings form in the neck, shoulder, belly or limbs, and is relatively benign. In the sheep, infection is generalized and runs a rapid course. In the pig, massive swellings form in the throat and neck, and recovery may follow.

The post-mortem signs are very similar in all these animals. The belly is distended, the blood is dark and tends to remain fluid, and putrefaction ensues rapidly. A bloody fluid may escape from the orifices of the body. The vascular system is engorged, and ecchymoses or larger hæmorrhages may be present in the tissues, organs and serous cavities. The lymphatic glands are enlarged, congested and hæmorrhagic. The spleen is notably affected. In the horse, ox and sheep it may be from four to eight times the normal size, dark in colour and engorged with blood, soft and friable ; the striking appearance of this organ in these animals gives origin to the common name of ' splenic fever ' applied to the disease. The liver is congested and friable, and the kidneys are congested and ecchymosed and the urine is almost always blood-stained.

The massive swellings in the external forms of anthrax infection develop around a lymphatic gland and consist of a hæmorrhagic gelatinous œdema infiltrating the muscular or other tissue of the part. Anthrax bacilli are abundant in it and also in the spleen ; in the other organs and tissues they are less numerous and occur almost entirely in the capillary blood-vessels, the arterioles and veins containing relatively few. Thus, in the kidney the bacilli are mostly confined to the glomeruli and capillaries between the tubules. The blood, organs and tissues, the nasal discharge, dejecta and urine are all infective, but it is the exception for the milk to contain bacilli.

Microscopically, the red blood corpuscles are irregular in shape and their contour is ill-defined, tissue fibres are swollen and œdematous, and muscle fibres and glandular and other cells show marked cloudy swelling or more profound degenerative changes.

The laboratory rodents—rabbit, guinea-pig, rat and mouse—are readily infected by cutaneous or subcutaneous inoculation of a trace of culture. Death ensues in the rabbit within about 48 hours, in the guinea-pig in 36 to 40 hours, and in the mouse and grey rat in 20 to 30 hours. The adult white rat is more resistant, but it, too, can generally be infected if large doses of culture are administered (Hall, 1912). Symptoms in these animals are inconspicuous, there is some fever, but only on the approach of death does the animal appear to be ill—it crouches in a corner with little disposition to feed or move, the coat may be 'staring', and convulsive attacks, dyspnœa, and general weakness supervene shortly before death. *Post mortem,* the inoculation spot is the site of a considerable gelatinous hæmorrhagic œdema, and the lymphatic glands draining this area are enlarged, congested and hæmorrhagic. The viscera are congested and engorged with blood, and the serous membranes may show hæmorrhagic spots. The most noticeable feature is the great enlargement of the spleen, which is dark, congested, soft and friable. Anthrax bacilli have the same distribution as in the larger animals.

PATHOLOGY OF ANTHRAX AND QUESTION OF TOXIN.

Various theories were formulated in the past to account for the manner in which the anthrax bacillus produces its effect, but these need not be discussed here. In modern times the tendency has naturally been to attribute the effects produced to toxic action. It seems reasonable to suppose that toxic effects do occur in anthrax, for frequently at an early stage after infection and before there has been dissemination of the bacilli, pyrexia with spreading œdema occurs. Attempts to obtain an exotoxin have been comparatively unsuccessful, and it is suggested that the anthrax bacilli break up in the tissues yielding a toxic substance; in other words, the hypothetical anthrax toxin belongs to those nebulous substances the endotoxins. Bail (see Bail and Weil, 1911) suggested that in anthrax infection there is an aggressin intoxication and that an anthrax immune serum is active by virtue of anti-aggressin. The alleged aggressins are obtained from the œdema fluid or pleural fluid of an infected animal after removal of the bacilli by centrifuging and the use of toluol.

Some of the attempts to obtain an anthrax toxin may be summarized. Brieger and Fränkel (1890) obtained a substance they termed a 'toxalbumin', and Hankin (1889), Hankin and Wesbrook (1892), and Peterman (1892) all found an 'albumose', which was not very toxic and seemed to possess some protective and immunizing power. Sidney Martin (1889–90) by cultivating the anthrax bacillus in an alkali-albumin medium also obtained an albumose and in addition an alkaloidal substance. Both were toxic and caused death, and he claimed to have recovered the same substances from the spleen and blood of animals dead of anthrax infection. The albumose caused fever, the nitrogenous body caused local œdema, enlarged spleen, and coma, but no fever.

Marmier (1895) also claimed to have obtained a toxin from cultures in fluid serum or in glycerin-peptone broth (free from albumose) grown at room temperature. The same toxin may also be obtained by extracting cultures grown on solid media. It is precipitated from the extract by sulphate of ammonium, the precipitate is taken up with water, the solution is concentrated *in vacuo* over sulphuric acid, precipitated again by alcohol, and the precipitate is washed in alcohol, and dried with ether *in vacuo.* Marmier states that this toxin acts upon animals as does the living bacillus, but finds that its action is not uniform towards individuals of the same species; a small dose may kill one rabbit, and a much larger dose of the same toxin may fail to kill another rabbit. The toxicity of this body is lowered but not abolished by heating it to 110° C. but is destroyed by the simultaneous action of air and sunlight. It is neither an albuminous body nor an enzyme. Rabbits can be immunized with it; when toleration to 30 mgm. is reached immunity against the living anthrax bacillus may have been acquired and may last 5 or 6 weeks. But mice and guinea-pigs could not be immunized in this way.

Boidin (1905) claimed to have obtained a toxic substance, which he regarded as a fat; this is of interest in view of the tendency of late to regard the toxins as being lipoid substances. This toxin was isolated by extracting the residue of filtered glycerin-peptone cultures with ether or chloroform, filtering the extract through a filter-candle and driving off the solvent by means of heat. It is insoluble in water but soluble in oils and can be emulsified in alkaline solutions. Introduced in its native state into an animal, it produces a local œdema similar to anthrax œdema; injected in oily solution it produces fatal general symptoms.

Although the striking similarity of the symptoms produced by Marmier's 'toxin' to those of clinical anthrax, and certain general pathological considerations favour the existence of anthrax toxins, the negative results of Conradi's (1899) experiments, viz. the introduction into the peritoneal cavity of broth cultures of virulent anthrax bacilli enclosed in collodion sacs that would allow the passage of toxins while holding back the bacilli, are curiously at variance with such a conclusion.

The existence of an anthrax toxin must, therefore, be regarded as being unproven, and this subject, as well as the general pathogenic action of *B. anthracis*, merit further investigation.

ANTHRAX IN MAN.

Three portals are open in man to the anthrax bacillus—the skin, the respiratory organs and the alimentary tract. A primary sore, the 'malignant pustule', develops in the skin and in the alimentary tract, and may do so in the respiratory organs also, but its presence cannot always be demonstrated. When the sore appears in the nose or at the edge of the larynx—both rare instances in the writer's (Eurich) experience—its primary nature is self-evident, but in the bronchi the evidence is not so clear. Local changes in the bronchi, analogous to those seen in the skin have been noted, but, as they have been found after general anthracæmia had been set up, they may have been the result of bacillary emboli, as are many of the hæmorrhagic necrotic foci found in stomach and bowel in generalized anthrax. It is possible that as in the experimental respiratory anthrax of rabbits, so the spores are phagocytosed in man also, and are carried by the phagocytes through the intact bronchial mucosa, and then, freed by the death of the carrier-cell, assume the vegetative form. Generalization is the rule in these internal types of the disease. In the skin, as a result of a less degree of vascularity, the changes remain localized much longer, and may indeed remain so confined, giving rise first to a small area of inflammation, like a flea-bite. This increases with varying rapidity, and, as it grows, the epidermis is raised to form either a single vesicle, or a number of vesicles which may coalesce. The texture of the fibrous tissue is loosened, the elastin of elastic fibres of the cutis is softened, capillaries and veins become dilated and thrombosed, infiltration with leucocytes takes place, and ' mast-cells ' and Unna's basket-cells appear. Very soon coagulation necrosis occurs, and thus a central black eschar is formed. The bacilli lie in greatest abundance immediately below this eschar and for a short distance beyond its lateral confines. They do not penetrate deeply in great numbers, but some may penetrate sufficiently deeply to escape the surgeon's knife when excision of the ' pustule ' is carried out, and may plug smaller veins of the corium and of the subcutaneous tissues. The infection is thus spread by way of the blood-stream and of the lymphatics. Wherever the bacilli linger, there they tend to bring about a hæmorrhagic exudate, especially in the lymphatic glands and in the stomach and bowels. The embolic origin of these foci in stomach and bowels is often evident from the clearly secondary changes on the surface of the mucous membranes, when compared with the more intense manifestations in their deeper layers, and from the ease with which bacillary emboli can be found. Though detection of the bacilli in the blood-stream is mostly an ominous sign, it is not necessarily so; symptoms of generalization may remain absent and recovery may ensue.

Accessory affections for which the anthrax bacillus is responsible are not known, though rheumatic pains are at times complained of in internal anthrax. The discovery of anthrax bacilli in an endocarditic vegetation is a pathological curiosity.

The mortality from respiratory and alimentary anthrax is very high; all cases (more than 30) seen by Eurich died, but instances of recovery from pulmonary anthrax have been recorded. Some writers claim a recovery-rate of 10 per cent. and even more; but in many of these cases a bacteriological diagnosis had not been made, and a mortality rate of

99 per cent. will be nearer the truth. The prognosis in cutaneous anthrax is very different; the mortality rate varies from 5 to 15 per cent.—these differences being due in part to varying facilities for an early diagnosis, in part to the occupation of the victims, certain industries favouring the localization of the pustule in areas such as the neck, from which generalization more readily takes place. The name 'pustule' is an unfortunate one, for pus formation is absent.

Post-mortem signs. The states of the various organs are for the most part similar after death from all forms of anthrax. The blood is almost always fluid and very dark in colour. Post-mortem discoloration appears rapidly and is of more than the usual lividity. Within two to four hours the lips, ears, neck, upper part of the chest, and shoulders are of a deep purple colour, the nails of a black-blue colour, and the skin of the penis and scrotum claret-coloured. Chocolate-coloured fluid may trickle from the mouth and nose.

The internal organs may be engorged, and have ecchymoses on their surface and hæmorrhages into their parenchyma. Extensive serous effusions, which may be more or less blood-stained, may be present in the pleural, pericardial, and peritoneal cavities. The external muscles of the chest are often dark-coloured, the heart-muscle is dark, soft and flabby. A gelatinous œdema behind the sternum is often found in pulmonary anthrax. The bronchial glands are generally enlarged, and in pulmonary anthrax the trachea and bronchi may contain a frothy blood-stained mucus and their mucous membrane be swollen and dark. In intestinal anthrax, the mesentery may show gelatinous œdema with hæmorrhages, the mesenteric glands be enlarged, and the intestine and stomach have patches of extravasated blood under the mucous membrane, which is swollen and may show large mulberry-coloured congested patches. The spleen is generally enlarged, dark and soft, but is sometimes unaltered in size and appearance. The liver is less frequently affected than other organs, but may be enlarged, engorged and hæmorrhagic. The kidneys may show small extravasations of blood under the capsule, and much blood may be present in the cellular tissue around them. Extravasations of blood are not infrequent between the membrane of the brain and spinal cord and into the lateral ventricles.

The gelatinous œdema of the cellular tissues of various parts of the body, if it has occurred, will persist after death.

Distribution of the anthrax bacillus. In man the bacillus tends to be less numerous in the blood, fluids and tissues than in animals. Even in a generalized infection none may be found in the blood at an early stage. The gelatinous œdema contains few bacilli, but the serous fluids generally contain numbers. In sections of the organs, bacilli will generally be found in small numbers, but are usually not numerous even in the spleen; occasionally they are innumerable. All fluids and tissues containing the bacilli if inoculated when fresh into a mouse or guinea-pig produce anthrax, but if kept for three or four days before inoculation may fail to infect.

Mechanism of Spread of Infection.

Many outbreaks of anthrax and numerous isolated cases of the disease are traceable to infected raw materials imported from foreign parts, especially from the East. Thus, in Saxony and in other parts of Germany there was, during the War, a cessation of the importation of foreign hides in consequence of the blockade, and with it a great fall in the incidence of anthrax, while a rise has again been observed since Germany's overseas commerce in these articles has re-developed. The infectivity of foreign animal products which generally reach this country in a dry state is possible only as a result of the intense vitality of the spores. Even the bacilli may survive drying for several weeks and in the dried state resist a heat more intense and acting for a longer period than do the bacilli in a fluid medium. While the former may survive exposure to a temperature of 90° C. for an hour and a half, the latter are killed at this temperature in a minute. Anthrax bacilli in liquid blood and serum are killed in half an hour at a temperature of 55° C. The spores are highly resistant to heat, cold and sunlight, but this resistance varies in different strains and according to their environment. In the dry state they tolerate temperatures over 100° C. and contact with formalin vapour sufficiently long to make this method of disinfection impracticable.

In soil anthrax spores are said to be able to retain vitality and virulence for 15 years. It is alleged by Graf that the infection of meadow and other land disappears as a result of the action of light and air—a statement at variance with the experience of graziers in this country. What special properties of the soil favour its growth and its persistence in it is not known, but damp, marshy, low-lying localities, especially if subject to inundations and if rich in decomposing vegetable matter, have long been known to have a bad reputation in this respect. Green food and dried fodder obtained from these tracts of land may convey the infection to animals, and these to man. It is possible that the animal which has yielded the infecting product is not necessarily itself infected. Gegenbauer (1920) states that anthrax spores become more numerous on damp, infected goat-hair at 20° C., and experimenting with white mice he found that spores attached to the hair in no instance caused death, though virulent bacilli could be cultivated from it. He concludes that a multiplication of bacilli and spore formation may take place on the hair of animals under natural conditions also. The animal may thus become a carrier (porter) ; but living carriers in the ordinary sense are not known ; the bacilli do not persist in animals which recover from the disease, and chronic infection is unknown.

Variations in virulence and toxicity of cultures have already been referred to ; but whether such variations occur in nature is not known, though highly probable. It has been remarked, though the observations are not sufficiently numerous, that anthrax in man, when contracted from East Indian wool, appears to be less severe than when contracted from other sources, such as Persian wool; it is possible, however, that the alleged difference is due to a more massive infection from the latter material rather than to infection with a more virulent stock.

The transference of the infection to man is usually one of direct contact with a diseased or fallen animal or with its products. Thus farmers, drovers and butchers, on the one hand, and fellmongers, tanners and operatives handling wool and hair, on the other, may contract the disease. Infection is mostly through the skin, and it is highly probable that a lesion, however small, must prepare the skin for infection, as animals with a more delicate skin such as guinea-pigs are said not to suffer if virulent anthrax spores be applied to a shaven surface, though it is true that mice succumb to the same process. It is a curious fact, however, that except in the case of farmers and butchers a history of a cut or other injury is rarely obtainable. Though the writer (Eurich) knows of no instance in which the disease was conveyed from man to man, a healthy person may be a carrier (porter) as regards his clothes; thus a schoolboy played football with factory hands still clothed in their overalls and contracted cutaneous anthrax; a woman became infected through washing her husband's ' brat ' (overall).

Cutaneous anthrax on the fingers is rare; tainted finger-nails may, however, convey the infection, or dust arising from the manipulation of wool or hair may settle on distant and exposed parts, such as face and neck. Or, again, the virus may be introduced by friction with a neckband or a towel. In tanneries, the worker's neck may come in direct contact with the infected hide as he carries it. As a result of the statutory precautions now taken in factories where wool and hair are handled, less dust is imparted to the atmosphere, and hence pulmonary anthrax or ' Woolsorters' Disease ' has become rare. Infected dust may, however, be derived from a worker's clothing; thus, a man who had left his work for a week took part in a street-fight and received a good ' dusting '; 24 hours later he was taken ill and died of pulmonary anthrax. As anthrax spores may pass through an insect's body unchanged, insect-bites may convey the disease, but authentic cases are rare.

As the processes of manufacture may dilute but rarely destroy the virus, the finished article may still convey the infection; thus it has been traced to shaving-brushes, blankets and cardigan jackets. Food is rarely the cause of anthrax in man in this country. Gipsies, seemingly less particular in their choice of food, and South African natives, have occasionally contracted anthrax in this way. Thorough cooking is probably the reason why infected meat that has reached the market so rarely gives rise to anthrax. That man has some degree of natural local resistance to anthrax is clear from the relatively low mortality from its cutaneous variety; but nothing is definitely known as to the effect of age or of constitutional defects or of modes of life upon the course of the disease, nor as to racial differences and the influence of climate.

As for pulmonary anthrax—we recognize only those cases in which the patients suffer severely; the true nature of milder cases (if they exist) may occasionally be suspected but is rarely proved. Whether resistance in man ever amounts to immunity is not known, and because of the seemingly random mode of convection of the infection an answer to this question is impossible at present. One attack of the disease, if it produces immunity, produces only a shortlived one; a second attack of cutaneous anthrax has been known to occur within a year. Nothing, too, is known regarding the break-down of resistance in man; just as grave interference with the normal condition of life of an animal is known to cause such a break-down, it may with good reason be argued that the same may take place in man; but what these unwholesome conditions may be—whether starvation or poisons (e.g. alcohol), or other unhygienic modes of life—is quite unknown.

Practical Diagnosis.

DURING THE COURSE OF THE DISEASE.

Here the diagnosis of anthrax is a bacteriological one. In the case of *cutaneous anthrax* the technique is so simple that any practitioner possessing a microscope of medium power and a few staining reagents can make the diagnosis provided he realizes the possibility of anthrax. Given a 'pustule' with its classical characters the thought of anthrax should arise naturally; but the diagnosis should be made earlier if the risks attending the disease are to be minimized. The patient's occupation will often supply the all-important clue. Recognizing this, the Bradford textile firms dealing with dangerous wools have appointed a medical officer to whom all workpeople presenting suspicious lesions of the skin are sent, and who may be asked to visit those the nature of whose sickness is not immediately obvious. If a vesicle has formed, films are made of some of the serum and stained. If a vesicle has not had time to develop, or is already broken, gentle scraping of the pimple or of the central denudation will produce a serum usually rich in bacilli. Their cultivation should in all cases be attempted (see also p. 446); but it may fail—especially if the serum has in transport been given time to exert its bactericidal power. When the serum has to be sent some distance it is best forwarded dried on silk threads or as large thick drops on slides or pieces of earthenware. Even the fully developed 'malignant pustule' should be examined bacteriologically as its appearance may be simulated closely by the vaccine pock and by some diplococcal and streptococcal infections.

In *pulmonary anthrax*, the bacillus has been found microscopically in sputum and in the pleural exudate, and in *gastro-intestinal* anthrax in the vomit.

In *animals* the discharges from nostrils and anus have yielded the bacillus—rarely the urine, except when blood-stained.

In the terminal stages of the disease the bacillus may be cultivated from the blood-stream if a sufficient quantity (20 c.cm.) of blood is taken. But the anthrax bacillus may be isolated from the blood-stream in non-fatal cases also, and in the absence of symptoms suggesting generalization.

The estimation of the *opsonic index* may prove useful in the hands of one who has acquired the necessary skill; a negative phase, even, is of diagnostic import and has been found in one case by Eurich to indicate a re-infection of the wound after excision of the pustule. For technical reasons the estimation is carried out by Eurich with free anthrax *spores*, as it is an open question whether similar results would be obtained with *bacilli*, for though non-capsulated bacilli are taken up readily by leucocytes of human-, horse- and rabbit-blood, bacilli, if capsulated, are said to be phagocyted only in the blood of an immunized animal (horse); and differences in the fate of bacilli appear to depend also upon their cultural or their animal origin (Combiesco, 1923).

Complement fixation has also been employed but is still on its trial as a means of diagnosis in this disease.

POST-MORTEM DIAGNOSIS.

As the post-mortem signs in man are neither constant nor absolutely characteristic, post-mortem diagnosis finally rests upon (*a*) the finding of the anthrax bacillus and (*b*) the precipitin-test of Ascoli.

Of the *microscopic detection* of the anthrax bacillus all that is needful has been said in previous paragraphs. Whenever possible it should be corroborated by cultivation, and by inoculation of one or more guinea-pigs or mice. If material has to be sent to a laboratory it should consist of the spleen, of blood dried upon a piece of rough sterilized earthenware from which it is less likely to break away during transit, of hæmorrhagic gland, and, in animals, of an ear. The writer (Eurich) has the impression that failure to find the bacilli is somewhat less frequent when agar plate cultures are used and if the material to be examined is dispersed in the melted agar before this is poured into the dish. Though anthrax bacilli are to be found *post mortem* in great abundance in the spleen, failure to cultivate the bacillus is common when the examination has been delayed, as this organ is one of the first to putrefy; it is then advisable to take blood also from a peripheral vein for cultural tests. Though anthrax bacilli may appear to be abundant under the microscope, one or two colonies only may develop.

Ascoli's thermo-precipitin test (Hoffmann, 1922), the principle of which has already been dealt with (p. 448), may help when putrefaction interferes with other methods. The precipitinogen furnished by the bodies of the bacilli being very resistant to heat, putrefaction and preservatives, the spleen or any organ or tissue sufficiently rich in bacilli may, even though in an advanced state of putrefaction, or after preservation in alcohol or formalin, still yield this body. Two agents are needed for the test: an extract containing the precipitinogen from the organ to be examined, and a specific serum. Immune sera used for protective or therapeutic purposes may or may not contain precipitins in sufficient amount, and the precipitating serum should, therefore, be specially prepared.

The method of applying the Ascoli reaction, which is fully described in Vol. IX, is to take a little of the organ extract—e.g. a fragment of spleen boiled with five to ten times its volume of saline, cooled and filtered—in a narrow test-tube and to introduce under it with a pipette some of the special precipitating immune serum. If the result is positive, a faint, thin, whitish turbidity forms at the junction of the two liquids immediately, which rapidly increases in amount and become flocculent. A series of controls is put up at the same time.

Ascoli and Valenti (1910) published the result of their first systematic examinations of the test in 1910; the spleens of 15 head of anthrax-infected cattle, positive to the usual laboratory tests, were tested successfully by this method in spite of advanced decomposition. Schütz and Pfeiler (1911–12) published corroborative evidence, having carried out tests on a large scale; in 40 out of 45 cases the results of the test and of bacteriological examination agreed; the five remaining cases were as follows: (1) autopsy two days after death—anthrax suspected—bacteriological evidence negative: Ascoli positive; (2) autoptic findings suspicious: microscopy negative and Ascoli negative; (3) anthrax-like bacilli found by veterinary surgeon, none found in laboratory, Ascoli negative; (4) autoptic diagnosis of anthrax, bacteriologically negative; Ascoli positive; (5) post-mortem diagnosis: sero-fibrinous peritonitis: microscopically, anthrax-like bacilli; cultivation negative, Ascoli negative. Szymanowski and Zagaja (1911) examined 68 cases: in 55 the thermo-precipitin test confirmed the bacteriological diagnosis: in 11 it gave a positive result when other methods had failed: in 2 it gave a negative result in spite of positive bacteriological findings; this was probably because of poverty of the tissue in precipitinogen, but the result might also be explained as being due to Flemming's (1912) paradoxical reaction. Further support has been furnished by the investigations of Zwick (1913), Fischöder (1913), Declich (1912) and others, both in animals and in man. While the spleen gives the best results in most instances, in pigs the material must be taken from the primarily infected organ or tissue in accordance with the known frequency of a purely local infection in this animal.

Though Ascoli's test is, broadly speaking, a group-reaction, it is not so in practice, if proper dilutions are employed. It must fail when the bacilli in the tissues examined are too few; thus it is more likely to be successful with the subcutaneous fibrous portions of hides than with their outer layers. Though its usefulness in the rapid detection of anthrax-infected hides has been proved, the margin of error is here possibly a wide one, for whereas of 38,000 Eastern hides only 0·5 per cent. gave a positive Ascoli, the anthrax bacillus could be cultivated by the writer (Eurich) in 14 per cent. of 14,000 Eastern wools. Pohl (1927), however, found the Ascoli test with hides to be quite trustworthy.

Irregular results are given by food-stuffs, and the Ascoli test possesses no advantages over the usual laboratory methods. Again, it is of greater utility for post-mortem diagnosis than during life. Thus Januschke (1922) has shown that an intra-vitam diagnosis cannot be made earlier by means of it than by cultural methods, as bacilli can be grown from the blood before their numbers are sufficient to give a positive Ascoli. Precipitinogen was demonstrated by him at the earliest 24 hours after experimental infection ; the reaction is usually indubitably positive only when bacteriæmia is advanced ; in peracute cases it gave a positive result almost synchronously with cultural methods, while in more protracted cases cultivation was successful 24 to 32 hours before the other. If intra-vitam diagnosis by means of the thermoprecipitin test is to be attempted a highly potent serum must be used.

Wilke (1919) successfully tested the fæces of anthrax-infected pigs and cattle with Ascoli's method when the infection was generalized, but not when, as in pigs, the disease was localized.

The utility of Ascoli's test in veterinary practice would be greatly restricted could it be shown that preventive inoculations give rise to a positive reaction : but this is not so, as repeated experiments by Bierbaum and Krause and by Francke and Standfuss, and others have proved. It does not appear to have been employed in the examination of wool and hair. In blood-stained samples of these it may be applicable, but as much wool, especially East Indian wool, has been washed before exportation, and as anthrax spores in it are often scanty, while those of allied organisms are very numerous, the precipitin test is little likely to give results superior to those obtained by cultivation.

The utility of the Ascoli test for the determination of the nature of anthrax-like bacilli in cultures is not yet definitely established. Senge (1913), as also Jänisch, found the test of service. Despite these limitations, which it shares with other sero-diagnostic methods, its utility is now fully recognized and the Governments of several States have made its application compulsory.

No great modifications of the method have been made by later workers. Francke *et al.* (1924) extract 1 gm. of hide cut up into pieces the size of a lentil or of a grain of rice, with 5 c.cm. of phenol-saline in the ice-chest for some hours and then centrifuge the extract instead of filtering it. Standfuss and Schnauder (1925) use small quantities—e.g. 0·05 gm. of tissue and 3 c.cm. of phenol-saline—they extract in the ice-chest and centrifuge for 10 minutes at a velocity of 1,000 revolutions per minute.

Immunization.

VACCINATION OF ANIMALS WITH CULTIVATED VIRUS.

Though anthrax among horses, cattle and sheep has a high death-rate it is not inevitably fatal. Cattle that survived an attack were found by Pasteur to be immune to infection with a highly virulent strain ; and so when it was seen that some sheep in la Beauce were immune it was conjectured that they owed their immunity to an attack of anthrax of a mild type. The existence of mild forms of the disease has since been proved, and it became Pasteur's aim to obtain an attenuated strain of constant virulence with which to produce a disease mild in type and leaving behind a serviceable degree of immunity. This attenuation was effected by incubating broth inoculated with anthrax-infected blood at 42·5° C., which had the double effect of preventing spore-formation and of producing a gradual loss of virulence, till on the eighth or tenth day the culture failed to kill guinea-pigs and rabbits. Sheep inoculated with such a culture survived and proved immune to a subsequent injection of a highly virulent culture. Subcultures from the attenuated virus could be grown at 37° C., and, though spores developed in them, these spores still produced bacilli of the same degree of virulence. As the degree of immunity attained depends upon the strength, i.e. virulence, of the vaccine, and as the more potent the vaccine the greater is the risk to life, it was found advisable to follow the injection of the avirulent vaccine (vaccine i) 14 days later with the injection of a more virulent one, called vaccine ii. Vaccine i consisted of a culture which would kill mice, but was harmless to rabbits, while vaccine ii would kill mice, guinea-pigs, and sometimes rabbits. The dose of each vaccine was the same : 0·25 c.cm. for cattle, and 0·125 c.cm. for sheep.

Age (i.e. youth), malnutrition, pregnancy and previous ill-health increase the risk of vaccination and probably account for a death-rate, the result of vaccination, of ¼ to ½ per cent. In order to determine whether death after vaccination is actually due to the vaccine, R. Kraus (1924) recommends that the bacillus obtained from the blood of the deceased animal be injected into rabbits ; as the virulence of the original strain is not increased in its passage through the sheep, death of the rabbit would prove that the vaccine was too virulent. To lessen this risk, small though it is, Sobernheim (1897, 1899) has recommended the combined use of immune serum and vaccine. His vaccines were of five strengths, for obtaining which he employed both low and high temperatures. They are : i, grown for 20 weeks at 12·5° C.—innocuous to rabbits, guinea-pigs and mice ; ii, grown for 20 weeks at 42·5° C.—innocuous to rabbits, sometimes noxious to guinea-pigs, always fatal to mice ; iii, grown for 11 days at 42·5° C.—fatal to guinea-pigs and mice ; iv and v, correspond to Pasteur's vaccines i and ii.

Marchoux (1895) had already investigated the protective power of immune sera but his results had not been encouraging. The immunity of sheep and rabbits induced by Pasteur's method had to reach a high degree before the sera of these animals could be used to protect others ; and animals treated with these sera might survive a first inoculation with virulent material but often succumbed to a second. The curative action of these sera was also slight ; of 24 rabbits to which the serum was administered 15 to 20 minutes after inoculation with anthrax only 7 survived, and these did not show themselves later to be immune. Though

Sclavo (1895) claimed that 2 c.cm. of his immune serum (obtained from the ass) will save a rabbit inoculated 12 to 24 hours previously with 1 c.cm. of anthrax culture, doubt has been thrown upon the specific immunizing properties of his serum—a doubt that is strengthened by the experiments of Rosatzin (1899), who showed that the normal serum of rabbits (whose susceptibility to anthrax was demonstrated subsequently) injected into guinea-pigs simultaneously with a large amount of a virulent anthrax culture prevented infection. Sobernheim, indeed, stated that specific immune bodies are contained neither in the serum of actively immunized animals nor in that of animals that have survived natural infection, a conclusion supported many years later by Kraus. He thought, however, that he had found indications of specific immunizing action in the serum of immunized rams; 4 c.cm. of this sufficed to prolong the life of infected rabbits—a protection which could, however, not be increased by an increase of dose. Later he combined the serum with vaccine ii (10 c.cm. serum with $\frac{1}{10}$ loop of vaccine ii), giving it for some months and obtaining thereby a degree of immunity in sheep which enabled them to tolerate the injection of enormous amounts of an anthrax culture, and even protected them against alimentary anthrax. As anthrax does not inevitably follow the introduction of anthrax spores with the food a large number of tests would be needed to prove the latter assertion.

Immunization by Pasteur's and by Sobernheim's method has been practised on a large scale in many countries. Hutyra (1898) (Hungary) reported that in the 5 years 1889–94, 7,837 horses were immunized according to Pasteur's method with a total loss of 0·19 per cent. by the end of the first year, 111,463 heads of cattle with a mortality of 0·1 per cent. and 445,823 sheep with a mortality of 0·62 per cent. Ssász (1918) reported that the combined method has been used in Hungary for the immunization of swine since 1914, with material prepared in the State laboratory at Budapesth; if the danger from infection is urgent, serum alone is given, its amount depending upon the size of the animal: 30 to 60 c.cm. may be given subcutaneously, but 10 to 20 c.cm. usually suffice, and an immunity is attained which he alleges will last 14 days. If the danger from infection persists, active immunization with vaccines i and ii follows—the dose in all cases, irrespective of age and race, being 0·1 c.cm. subcutaneously. To be effective vaccines must be fresh, for they tend to become weaker within a year, and many commercial vaccines have been found to be ineffective when tested on smaller animals.

Pasteur's method produces fever up to 41° C. in cattle, and also œdema, whereas simultaneous inoculations with vaccine and antiserum give no reaction either on the first occasion or on repetition of the procedure six months later. Anaphylactic symptoms have been frequently observed upon a repetition of Sobernheim's method; they may be strong if horse-serum has been used on both occasions, milder if sheep's serum has been employed; but even the most serious symptoms disappear without untoward results in a short time. Cows and heifers seem to be more susceptible than oxen. Gerlach's (1921) experiences of anaphylaxis in cattle suggested to him the advisability of using a serum for first inoculations only. Sero-immunization alone is of use only when the tide of an epidemic is to be stemmed ; in districts in which anthrax is a frequent or constant danger, hyperimmunization is advocated by Hruska—viz. the repetition of vaccine ii in a dose of 1 c.cm. Even such hyperimmunization may fail to protect some animals. Eichhorn (1925–6) reports similar experiences from the United States. After frequent failures, even with hyperimmunization, he obtained more satisfactory results when he used a strain which would produce definite local and general reactions—which he thinks desirable for the attainment of a satisfactory degree of immunity. These reactions are not usually dangerous.

Another explanation of failure may be found in Besredka's (1920, 1921, 1922) hypothesis that the virus is innocuous unless it can infect the cutaneous receptor-cells, and that immunization is effective when these receptor-cells are immunized, as with them the whole organism is immunized. This hypothesis is based upon the following observations: If vaccine i is applied to the shaven skin of a guinea-pig (or rabbit), or injected intracutaneously, a local reaction is produced from which the animal recovers ; but if vaccine ii is similarly applied the guinea-pig will die as a result of a local reaction and subsequent septicæmia. If, however, vaccine ii is applied in this way to the skin of a guinea-pig previously so treated with vaccine i, the animal not only survives, but will be immune to subcutaneous injections. Yet the serum of the cuti-infected guinea-pig contains no antibodies capable of protecting the young animal from infection. This susceptibility of the young animal depends, according to Besredka, upon the susceptibility of its skin, while other organs are refractory ; and hence a purely local immunization suffices to protect the animal against a general infection.

Besredka's results have been verified in many quarters. Thus Brocq-Rousseu and Urbain (1923, 1924) found that dead anthrax bacilli if injected intradermally into horses produced immunity, though no antibodies such as agglutinins and precipitins, were found in the serum of these animals subsequently, and no immunity could be conveyed by it to guinea-pigs. Sachelarie (1925) vaccinated 5 young beasts intracutaneously and 4 subcutaneously ; 3 of each group and 2 controls were inoculated a month later with 0·25 to 0·5 c.cm. of a 24-hour peptone-broth culture of anthrax—the former were the better protected. Another beast of each group and one control were fed with a mixture of anthrax spores, oats and splinters of glass : the control died, the others survived. The fifth of the intracutaneously treated beasts received an injection of a whole slope culture of a sporulating strain and recovered after 48 hours' fever and malaise. Velu (1924, 1927) reports that a single intradermal injection of vaccine, though it cause no thermal, local or general reaction, suffices to produce a solid and intense immunity, almost explosive in its development ; 1/5 of the minimal dose necessary in subcutaneous vaccination is effective if applied intradermally; and animals so treated survive the injection of 1,000 m.l.d. of virulent anthrax culture, and comport themselves in infected zones like animals that have been immunized by 2 or 3 subcutaneous injections of vaccine.

The claim that the method is applicable to all animals is supported by Nevodoff (1926) who adopted Besredka's technique (using vaccine i) for the vaccination of 743 horses and 3,349 head of cattle with excellent results; by Tatin and Velu (1926) who state that they have successfully immunized 1,300 horses by means of but one intracutaneous injection of vaccine: and by Nicolas (1926) who used it on horses and mules in Syria in 1925, and found the method harmless and productive of a satisfactory degree of immunity of one year's duration.

In spite of these favourable reports there is no consensus of opinion in favour of Besredka's views and results. Opponents contradict not only Besredka, but sometimes each other. Thus Tada (1924) found that guinea-pigs treated with vaccines i and ii—some subcutaneously, others cutaneously (by inunction of the shaven skin) developed immunity in equal proportions, viz. about 50 per cent., and he obtained similar results with intramuscular injections—a greater success with subcutaneous immunization than is usual in guinea-pigs.

Lastly, attempts have been made to produce immunity with non-specific substances other than sera. They are not numerous. Perhaps the most interesting are Turro's (1919) experiments if they can be verified; the contents of hen's eggs when beaten up constitute a medium favourable to the growth of the anthrax bacillus, but if ammonia is added, ferments are formed which largely disintegrate the bacilli. The addition of such ferments to a suspension of anthrax bacilli does not protect rabbits from infection, but animals treated with 'oviserum' are said to be immune to anthrax infection, and their serum to possess greater bacteriolytic power than normal serum.

Serotherapy and Chemotherapy.

Methods of active immunization have not yet been applied to man, and if adopted would have but a very limited application. Serotherapy and chemotherapy have, however, taken a recognized place in the treatment of human anthrax, and as regards the malignant pustule serum treatment is now the method of choice, taking the place of excision.

Serotherapy. Anti-anthrax serum is prepared by the intravenous inoculation of vaccinated animals with increasing doses of anthrax culture. Brocq-Rousseu, Staub and Urbain (1926) describe another technique for the preparation of anthrax serum. A horse is given several series of intravenous injections of increasing doses (from 10 cgm. to 30 cgm.) of an asporogenous anthrax culture killed with an alcohol-ether mixture. The animal is then given the two Pasteurian vaccines intradermally, and is finally treated with the alcohol-ether antigen intradermally. Several sera are now available—Sclavo's, obtained from asses, Sobernheim's, obtained from sheep (see p. 466), Mulford's, Parke Davis's, and others. For the standardization of anti-anthrax serum, Lusztig (1926) recommends the use of young white rats of about 100 gm. weight. A ' test dose ' of anthrax culture which kills by intraperitoneal inoculation in 4 days is ascertained. For the test, 8 rats are inoculated with the test dose of culture ; one of them is kept as a control, a second receives 0·3 c.cm. of normal horse-serum, and the remaining 6 receive the anthrax-serum to be tested—0·05, 0·08, 0·1, 0·15, 0·2, 0·3 c.cm. respectively—and the smallest dose of serum which will protect the animal is ascertained. The serum is given intravenously. If 0·1 c.cm. of serum prevents death, the serum is described as having a value of 1,000, since 1·0 c.cm. of the serum would protect 1,000 gm. of rat against the test lethal dose of culture.

Sclavo's serum has been the most extensively used with seeming success in cutaneous anthrax. Its value has been variously estimated. Sclavo himself lost only 3 cases out of 160 ; and Danielovic, having treated 78 cases with only 3 deaths, declares its efficiency to be equal to that of anti-diphtheritic serum, while Eurich has been less fortunate, with a mortality of 5 per cent. out of 300 cases. Sabolotnyĭ (1926) obtained recovery in all of 27 cases of human anthrax without general infection. In the earlier cases injections of 30 to 40 c.cm. of serum were given daily for 2 or 3 days, usually subcutaneously, occasionally intravenously. In the later cases a single injection of 100 c.cm. intravenously was the routine. Of 7 cases with generalized infection, 3 were not treated with serum and all died, 4 received serum treatment and 3 recovered.

Hodgson (1928) treated 31 cases of cutaneous anthrax with only 2 deaths. The two fatal cases were, however, moribund and died 15 hours and 4 hours respectively after admission to hospital. The serum was administered intravenously in doses of from 30 to 40 c.cm. to 100 c.cm., with a total of from 80 to 840 c.cm. for a case. One case, which ultimately recovered, is of interest. The lesion was situated in the upper arm, and after 300 c.cm. of serum had been given a re-infection lower down the same arm occurred.

Some have denied the value of anthrax-serum as a *specific* serum. Kraus and Beltrami (1921) have stated that the injection of *normal* ox-serum will give equally good results, and Penna (1918) treating 372 cases solely with ox-serum lost only 6·3 per cent. of his cases. Gerlach (1920), on the other hand, failed to corroborate Kraus's results. They have also been contradicted by von Hutyra and Manniger (1919), in whose opinion the normal sera of oxen and sheep are of less value than immune sera in protecting young rabbits against anthrax ; and by Kolmer, Wanner and Köhler (1920) who obtained similar results. Three factors, if unconsidered, will invalidate the results of a general comparison of statistics in man ; these are : the site of the pustule, the stage attained when treatment was begun, and the source of the infection. The importance of the first two is obvious ; the third has already been touched upon in an earlier paragraph. It is possible that Sclavo's exceptionally good results may be due to the fact that many of his cases arose from contact with pig's bristles.

An interesting contribution to the treatment of cutaneous anthrax is made by K. H. Erb (1926) : two men infected themselves simultaneously from the same source—one on the thigh in two places, the other at the bend of the elbow. In the first case the man's own blood was injected so as to form a tense ring around the pustule. The symptoms subsided more quickly in him than in the other. When a similar blood barrier was produced in mice after inoculation of the tail a fatal result was either delayed or prevented ; the varying susceptibility of mice must, however, be taken into consideration.

Chemotherapy deserves more attention than it has hitherto received. Salvarsan was recommended by Ehrlich and was tried by Eurich in 1910, and its congeners since then have been used with success by himself and others. Meshtschaninoff (1925) treated 21 cases with '914'. The temperature may rise precipitately after the injection, then often falls critically in 24 to 36 hours ; the œdema remains localized and quickly disappears and the pustule dries and heals without suppuration. A full dose is necessary. Erilow and Golotina (1926) added neosalvarsan to broth in proportions of 1 : 1,500 and 1 : 5,000, corresponding to the concentrations in the blood of man after doses of 0·3 to 0·8 gm. have been given, and found that this addition will kill spore-free bacilli ; 100 lethal doses treated for 3 hours with neosalvarsan yielded no cultures and failed to infect. Microscopic examination of the virus showed, according to length of action, changes in tinctoral characters of the bacilli, destruction of the bacilli, and finally their complete lysis. Added to human serum instead of to broth a similar action of neosalvarsan was observed : but when defibrinated blood was substituted, or a suspension of red corpuscles in sheep's serum, the action of the drug was much reduced, a reduction that could be corrected by a greater concentration.

Few other drugs have been tested, and these imperfectly. Möslinger (1924–5) found that argochrom strongly inhibited the vegetative forms in a dilution of 1 : 5,000 to 1 : 10,000—and faintly in a dilution of 1 : 200,000. Its clinical efficacy appears to have been established by Baumann (1922) in two severe cases, and by Eurich in one mild one. Auramin (of British Dyes, Limited), tested by A. Renshaw (Manchester) (in a personal communication to the writer, Eurich) as to its effects on pyogenic organisms, and neutral solutions of acriflavine are, on grounds of analogy, worthy of a trial. Burke and Rodier (1923) treated several of a flock of eleven sheep that were ill with acriflavine, others with methyl violet, in doses of 7 mgm. per kgm. of body weight in a 1 per cent. solution, saving all the animals but one. Attempts have also been made to treat anthrax infection in man with *B. pyocyaneus* and pyocyanase. Fortineau and Fortineau (1914) for instance treated 50 cases of human anthrax with injections of 10 to 20 c.cm. of sterilized broth cultures of *B. pyocyaneus*, with a mortality of 10 per cent.—not a very satisfactory result.

Antibacterial Measures.

DISINFECTION.

The action of light upon anthrax bacilli and their spores has been considered in a preceding paragraph. The only chemical disinfectants of value are formaldehyde and bichloride of mercury. Their activity *in vitro* upon naked anthrax spores in watery suspension is misleading, as, when applied in practice, they encounter conditions of many kinds whereby their bactericidal power is restricted and even prevented.

The conditions are simplest when the infected material is linen or cotton clothing that will not be damaged by boiling water or by steam, and when the contaminating substance which contains the bacilli or spores, such as dried blood or serum, is thin enough to be readily penetrated. Some raw materials, such as wool, are, however, irretrievably damaged by water above 60° C.—some even by 50° C.—and by steam ; or infected blood-clots may be so large and compact that their penetration is well-nigh impossible under ordinary conditions. Wool and similar products, such as alpaca and mohair, reach this country press-packed, and horsehair is tied in bundles so firmly, that only by hydraulic pressure could some degree of penetration be ensured, and even then insufficient to procure complete disinfection. Hence the bales and the individual fleeces contained in them must first be opened out—and bundles of horsehair cut open—processes that entail risk of infection, a risk that is lessened by the use of fans insisted upon by Government regulations in certain industries. Hides present the same difficulty of penetration.

The difficulties encountered in the case of wool, hair and bristles are best met by the method devised and recommended by the Home Office Departmental Committee on Anthrax (1919) and favourably reported on by Delépine : to soften and break up clots and excrement and to free imprisoned air the material is treated for 20 minutes in a vat containing a warm (46° C.) soap solution, after which for 10 minutes in warm water ; it is then passed through two successive baths of warm (38 to 39° C.) 2·5 per cent. solution of formaldehyde, each bath lasting 15 minutes. Between each of these steps, the material is passed between rollers, and again when it leaves the last formaldehyde bath on its way to a drying chamber. Here it is subjected to a current of hot air (71 to 75° C.), and remains for 25 minutes ; it is then stored for several days before it is distributed for use. The half-hour's sojourn in formaldehyde is not sufficiently long to kill all anthrax spores, but the action of the disinfectant begun in the baths is continued after drying, as this process leaves enough formaldehyde anchored to the spores to ensure their death during the period of storage. The cost of the method is not prohibitive ; it was calculated to be—at pre-war prices—about 0·8 penny per pound ; but the cost may be reduced if a method for recovery of formaldehyde can be devised.

Other methods and other substances have been recommended. Hydrochloric acid in a strong solution of sodium chloride can kill anthrax spores, but the slowness of its action and the loss of acid, consequent upon the frequent presence in the wool of considerable quantities of lime and other salts, prevent its use in the wool industry. Sulphurous acid, hypochlorous acid, and others will also kill, but they cannot be used, either because their action is too slow, or because, like hypochlorous acid, they injure the material.

A drawback to the use of bichloride of mercury is the formation by it of insoluble albuminates—a difficulty that may be overcome by the addition of, or the preliminary application of, an acid such as hydrochloric or formic acid. Upon this is based the Seymour-Jones method of disinfecting hides. Dried hides and skins are converted into 'wet-salted' hides by soaking them for 24 hours in a 0·5 to 1·0 per cent. solution of formic acid and then placing them in a bath of brine. To the formic acid 'soak' bichloride of mercury to the strength of 1 : 5,000 is added, which it is claimed will be sufficient to kill all spores of anthrax—a claim which C. Ponder (1911) confirms. Gegenbauer (1918), however, reported unfavourably upon the method, finding that anthrax spores escaped even after 14 days' treatment. He thinks that the alleged favourable results were due to insufficient removal of the bichloride before cultures were made. Hewlett also considers that the mercuric chloride used in the Seymour-Jones process needs to be in a strength of 1 : 1,000. Hewlett and Hall (1911) emphasized the importance of using agar culture media, and not broth, in experiments upon the disinfection of anthrax spores, the former often giving growths when the latter gives none and indicates sterility.

In the case of horse-hair, the Home Office admits the use of certain precautionary measures, such as the soaking of the material in a bath of disinfectant under prescribed conditions, as certified by a competent bacteriologist. The disinfectants employed for this purpose have usually been solutions of high-coefficient proprietary phenoloid disinfectants, and though as shown by Hewlett and Hall the process does not actually kill all the anthrax spores, it does appear to diminish the risk of infection.

In Germany, and to a less extent in Great Britain, hair and bristles were sometimes treated by current steam at a temperature not exceeding about 220° F., or by boiling in water for 2 hours. The damage caused to coloured hair by these processes is not material, though there is a tendency to make the fine ends brittle. White hair is, however, discoloured, and the elasticity of fine hairs is destroyed. For the last-named substances, boiling for one quarter of an hour in a 2 per cent. solution of potassium permanganate and subsequent bleaching in 3 to 4 per cent. solution of sulphurous acid may be employed. Anthrax spores vary considerably in their resistance to heat. Reiter (1920), using current steam, observed a survival of 5 minutes and 32 minutes as extremes with different strains of anthrax.

It is important that the carcasses of anthrax-infected animals should be disposed of in such a manner as to prevent the future dispersal of infection. If anthrax is suspected as a cause of death, no post-mortem examination should be made, but an ear should be cut off and forwarded to a laboratory for confirmation of the diagnosis. The carcass is best disposed of by burning, but such a procedure is usually impracticable. If, however, the carcass is buried in a deep pit with quicklime, little danger will ensue. The bacilli being confined in the body out of access of air will soon degenerate and die. Andrjevski (1928), finding that hydrogen sulphide is an active germicide both for the bacillus and for its spores, suggests that the carcass may be sprinkled with powdered iron sulphide, then well moistened with dilute hydrochloric acid, and finally covered with earth.

If the disease has not been suspected and a *post mortem* has been performed, similar measures of burial should be adopted, and all offal should be burnt, and the greatest care taken to swab all blood-stains, &c., with an efficient disinfectant, such as a strong solution of chloride of lime. Stalls or buildings in which cases of anthrax have occurred should be limewashed, and any bedding and the like burnt. It must not be forgotten that the affected animals before death may have had discharges from the mouth and rectum, and these must be looked for and disinfected.

ADMINISTRATION.

Outbreaks of anthrax among animals in this country are due for the most part to infected food-stuffs, such as grain which had been in contact with infected hides in the holds of ships, Bombay oil-cake, or turnips tainted with the blood of a slaughtered animal. Manures derived from the dust extracted from oriental and other foreign wools or mixed with foreign (often Indian) bone-meal may contaminate meadows and pastures. While the Governments of Australia and of New Zealand have passed stringent regulations of wide compass forbidding the importation of materials likely to be infected with anthrax unless thoroughly disinfected and certified as such by a responsible authority, and though the great reduction of anthrax during the war in the countries affected by the blockade has shown the utility of such restrictions, the only restrictive regulations at present in force in Great Britain deal with the importation of meat and the compulsory disinfection of Egyptian wool and East Indian goat-hair by an Order in Council. This disinfection (by means of formaldehyde) is carried out at present at the Government Wool Disinfecting Station, Liverpool. The list of materials the disinfection of which is advisable is increasing. Their alleged geographical origin is not always to be depended upon, and it may be safely assumed that all Eastern and African wool and hair, together with some South American products, are dangerous, though the extent of that danger may still have to be determined in some instances. This statement does not apply to Australian and New Zealand wool.

Anthrax, both in animals and in man, is a notifiable disease.

In the case of animals the duty falls upon the owner of the animal or carcass, upon the butcher, or upon the veterinary surgeon—the notification being made by virtue of the Anthrax Order or the Notification of Animal Diseases Order, to an inspector of the local authority, who must report to the Medical Officer of Health. The report finally reaches the Board of Agriculture and Fisheries. For details of the further rules respecting the procedure consequent on the examination by the veterinary inspector of the Board, the reader is referred to the Anthrax Order of 1910.

Although by the provisions of this Order the danger of the spread of anthrax from actual cases is much restricted, the pollution of rivers and streams and of drains and sewage-farms by the effluents of factories is not prevented. As Reddie, of the Bradford Sewage Department, and Eurich have shown, the water from wash-bowls, or discharge from tanneries, may carry anthrax spores in considerable numbers into streams and sewers, and thence on to adjoining land ; wool-dust escaping from dust-extractors into the open air has carried the infection on to pastures ; and wool-dust, its dangers all unsuspected, has been used as a valuable manure, e.g. in the hopfields of Kent. How the risk of infection from these effluents and waste products can be most effectively and economically prevented—except it be by the preliminary disinfection of the raw material—has not yet been determined.

In human anthrax the case is notified to the Certifying Factory Surgeon and to the Factory Department of the Home Office if the patient has contracted the disease in a factory or workshop, and, voluntarily, to the local Health Authority. The duty of notification falls upon the practitioner who sees the patient and recognizes the disease, and upon the employer as soon as he is informed of the case.

As regulations to minimize the danger from anthrax—such as the Woolcombing Regulations and those dealing with the manufacture of horse-hair—have been drawn up and have from time to time been amended by the Home Office, the circumstances of each case are enquired into by the local Inspector of Factories, infringements of these regulations are reported, and samples of the materials handled by the patient are examined bacteriologically. The regulations classify, in schedules, according to the estimated degree of danger, the various kinds of wool and hair which are especially noxious. The list of noxious wools might well be amplified, such materials as, for example, Mediterranean (Tunisian, &c.) hair, and Chinese wool not being included. According to the estimated degree of danger the regulations prescribe measures of varying severity, the main intent of which is to minimize the amount of dust raised in the process of opening bales and fleeces, or sorting, and of washing and combing. An additional and voluntary precaution is taken by some firms by offering a bonus to openers and sorters for the detection and removal of blood-stains on wool and hair, as such blood-stained specimens may contain anthrax spores in great abundance. A copy of the regulations and a monitory placard, with illustrations of cutaneous anthrax in various stages, are posted in every such factory.

REFERENCES.

General :

Annual Reports of the Chief Inspector of Factories, Home Office, London (H.M. Stationery Office).

BELL, J. H. & LEGGE, T. H., 1906, Article *Anthrax* in *System of Medicine* Clifford Allbutt, **2**, Pt. I, 227.

Report of Home Office Departmental Com. on Anthrax, 1918 and 1919 (Lond.).

ABT, G., 1921, *C.R. Soc. Biol.*, Paris, **84**, 627.

AÏTOFF, MARGUERITE, 1922, *Ann. Inst. Pasteur*, **36**, 567.

ANDRJEVSKI, P., 1928, *J. Bact.*, **16**, 151.

ARLOING, S., 1885, *C.R. Acad. Sci.*, Paris, **101**, 511.

ARLOING, 1921, cited by Besson, in *Technique microbiologique.*

ASCOLI, A. & VALENTI, E., 1910, *Z. InfektKr.*, **7**, 375.

BACHMANN, A., BELTRAMI, P. & ROMAT, A., 1923, *Rev. Asoc. med. argent.*, **36**, 631.

BAIL, O. & WEIL, E., 1911, *Arch. Hyg.*, Berl., **73**, 218.

BALTEANO, L., 1922, *Ann. Inst. Pasteur*, **36**, 805.

BAUMANN, E., 1922, *Klin. Wschr.*, **1**, 2472.

BESREDKA, A., 1920, *C.R. Soc. Biol.*, Paris, **83**, 769 ; 1921, *Ann. Inst. Pasteur*, **35**, 421.

BESREDKA, A. & DE TREVISE, Y., 1922, *Ann. Inst. Pasteur*, **36**, 562.

BOIDIN, L., 1905, *Arch. méd. exp.*, **17**, 695.

BORMANS, A., 1896, *Riv. Igi.*, **7**, 53.

BOUCHARD, CH., 1889, *C.R. Acad. Sci.*, Paris, **108**, 713.

BRIEGER, L. & FRÄNKEL, C., 1890, *Berl. Klin. Wschr.*, **27**, 268.

BROCQ-ROUSSEU & URBAIN, 1923, *C.R. Soc. Biol.*, Paris, **89**, 20, 487, 782 ; 1924, *ibid.*, **90**, 4, 1307.

BROCQ-ROUSSEU, STAUB & URBAIN, A., 1926, *Ann. Inst. Pasteur*, **40**, 595.

BUDD, W., 1863, *Brit. Med. J.*, i, 85, 110, 159, 237, 316, 557.

BURKE, V. & RODIER, E. A., 1925–6, *J. Amer. Vet. Med. Ass.*, **68**, 232.

BUZNA, D., 1925, *Z. InfektKr.*, **28**, 267.

CHAMBERLAND, CH. & ROUX, E., 1883, *C.R. Acad. Sci.*, Paris, **96**, 1088.

CHAMBERLAND & ROUX, 1921, cited by Besson, in *Technique microbiologique.*

CHAUVEAU, A. & PHISALIX, C., 1895, *C.R. Acad. Sci.*, Paris, **120**, 801.

CHURCHMAN, J. W., 1927, *J. Exp. Med.*, **46**, 1007.

COMBIESCO, D., 1923, *C.R. Soc. Biol.*, Paris, **89**, 634, 637 ; 1924, *ibid.*, **90**, 752.

CONRADI, H., 1899, *Z. Hyg. InfektKr.*, **31**, 287.

v. DARÁNYI, J., 1927, *Zbl. Bakt.*, Abt. I, Orig., **102**, 326.

DECLICH, M., 1912, *Z. InfektKr.*, **12**, 434.

DOLD, H. & WEYRAUCH, F., 1924, *Z. Hyg. InfektKr.*, **103**, 150.

DUCLOUX, E., 1920, *C.R. Acad. Sci.*, Paris, **170**, 1527 ; 1924, *ibid.*, **179**, 510.

DUPOND, R., 1905, *C.R. Soc. Biol.*, Paris, **58**, 911.

EICHHORN, A., 1925–6, *J. Amer. Vet. Med. Ass.*, **68**, 276.

EMMERICH, R. & LÖW, O., 1899, *Z. Hyg. InfektKr.*, **31**, 1.

EPPINGER, H., 1894, *Die Hadernkrankheit*, Jena.

ERB, K. H., 1926, *Beitr. klin. Chir.*, **137**, 202.

ERILOW, A. P. & GOLOTINA, Z. J., 1926, *Z. Hyg. InfektKr.*, **105**, 509.

EURICH, F. W., 1912, *J. Path. Bact.*, **17**, 249 ; 1927, Anthrax, *Med. Annu.*, Bristol, p. 38.

FISCHÖDER, 1913, *Berl. tierärztl. Wschr.*, **29**, 640, 658, 675.

FLEMMING, A., 1912, *Deuts. tierärztl. Wschr.*, **20**, 81.

FORTINEAU, L. & FORTINEAU, C., 1914, *C.R. Acad. Sci.*, Paris, **158**, 1035.

FOTH, H., 1919, *Deuts. tierärztl. Wschr.*, **27**, 348.

FRANK, G., 1899, *Münch. med. Wschr.*, **46**, 282.

FRANCKE, G., STANDFUSS, R. & SCHNAUDER, F., 1924, *Arch. wiss. prakt. Tierhlk.*, **51**, 530.

FÜRST, TH., 1920, *Arb. GesundhAmt.*, Berl., **52**, 93.

GEGENBAUER, V., 1918, *Arch. Hyg.*, Berl., **87**, 289 ; 1920, *ibid.*, **89**, 202.

GERLACH, F., 1920, *Zbl. Bakt.*, Abt. I, Orig., **84**, 396 ; 1921, *Berl. tierärztl. Wschr.*, **37**, 32.

GÓZONY, L. & KRAMÁR, E., 1922, *Zbl. Bakt.*, Abt. I, Orig., **89**, 193.

GRAF, P., 1926, *Münch. med. Wschr.*, **73**, 1888.
GRATIA, A., 1924, *C.R. Soc. Biol.*, Paris, **90**, 369.
GRIERSON, A. M. M., 1928, *J. Hyg.*, Camb., **27**, 306.
GUNDEL, M., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 463.
HAAG, 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 222.*
HALL, G. N., 1912, *Zbl. Bakt.*, Abt. I, Orig., **66**, 293.
HALLERMANN, A., 1925, *Zbl. Bakt.*, Abt. I, Orig., **96**, 419.
HANKIN, E. H., 1889, *Brit. Med. J.*, ii, 810.
HANKIN, E. H. & WESBROOK, F. F., 1892, *Ann. Inst. Pasteur*, **6**, 633.
HENRI, V., 1914, *C.R. Acad. Sci.*, Paris, **158**, 1032 ; **159**, 340, 413.
HESS, H., 1920, *Arch. Hyg.*, Berl., **89**, 237.
HEWLETT, R. T. & HALL, G. N., 1911, *J. Hyg.*, Camb., **11**, 473.
HODGSON, A. E., 1928, *Lancet*, Lond., ii, 594.
HOFFMANN, R. S., 1922, *Die Thermopräzipitin Reaction*, Wien. u. Leipzig.
HOLMAN, W. L., 1922–3, *Proc. Soc. Exp. Biol.*, N.Y., **20**, 60.
HRUSKA, C., 1928, *C.R. Acad. Sci.*, Paris, **186**, 1868.
v. HUTYRA, F., 1898, Quoted in *Ergebn. allg. Path. path. Anat.*, 1898, **5**, 581.
v. HUTYRA, F. & MANNINGER, R., 1919, *Zbl. Bakt.*, Abt. I, Orig., **83**, 518.
JANUSCHKE, E., 1922, *Z. InfektKr.*, **23**, 60, 123.
JOHNE, A., 1892–3, *Deuts. Z. Thiermed.*, **19**, 244 ; 1893–4, **20**, 73.
KATZU S., 1925, *Zbl. Bakt.*, Abt. I, Orig., **94**, 165.
KERN, F., 1897, *Zbl. Bakt.*, Abt. I, **22**, 166.
KOCH, R., 1877, *Beitr. Biol. Pfl.*, II, Heft **2**, 277.
KOLMER, J. A., WANNER, D. C. & KOEHLER, M. E., 1920, *J. Infect. Dis.*, **26**, 148.
KRAUS, R., 1924, *Seuchenbekämpfung*, **1**, 47.
KRAUS, R. & BELTRAMI, P., 1917, *Conf. Soc. sud-. amer. hig.*, B. Ayres, **1**, 151 ; 1917, *Wien. klin. Wschr.*, **30**, 553 ; 1921, *Z. ImmunForsch.*, Tl. I, Orig., **31**, 93.
KRISTENSEN, M., 1927, *Zbl. Bakt.*, Abt. I, Orig., **101**, 220.
LAUBENHEIMER, K., 1911–12, *Z. Hyg. InfektKr.*, **70**, 321.
LÜPKE, 1895, *Deuts. tierärztl. Wschr.*, **3**, 23.
LUSZTIG, A., 1926, *Zbl. Bakt.*, Abt. I, Orig., **98**, 492.
LUTZ, G., 1922, *Z. Hyg. InfektKr.*, **97**, 12.
MARCHOUX, E., 1895, *Ann. Inst. Pasteur*, **9**, 785.
MARMIER, L., 1895, *Ann. Inst. Pasteur*, **9**, 533.
MARTIN, S., 1889–90, *19th Rep. Med. Off. Loc. Govt. Bd.*, p. 235 ; 1890–91, *20th Rep. Med. Off. Loc. Govt. Bd.*, p. 255.
MARTOS, G. Z., 1927, *Z. Hyg. InfektKr.*, **107**, 477.
MESHTSCHANIPOFF, 1925, *Pr. Méd.*, **33**, 901.
METCHNIKOFF, E. & ROUX, E., 1891, *Ann. Inst. Pasteur*, **5**, 479.
MEYER, 1920, *Zbl. Bakt.*, Abt. I, Orig., **85**, 177.
MONTEIRO, J. LEMOS, 1925, *C.R. Soc. Biol.*, Paris, **93**, 975.
MORIHANA, S., 1921, *Zbl. Bakt.*, Abt. I, Orig., **86**, 405.
MÖSLINGER, A., 1924–5, *Vet. Med. Dissert.*, Wien.
NEVODOFF, A. P., 1926, *C.R. Soc. Biol.*, Paris, **94**, 170.
NICOLAS, E., 1926, *C.R. Soc. Biol.*, Paris, **94**, 336.
PANTON, P. N. & BENIANS, T. H. C., 1925, *Brit. J. Exp. Path.*, **6**, 146.
PASTEUR, L., 1878, *Bull. Acad. Méd.*, Paris, 2 s., **7**, 253 ; 1880, *ibid.*, 2 s., **9**, 682.
PENNA, J., 1917–18–19, *Prensa méd. argent.*, IV and V. ; 1918, *Rev. Inst. bact.*, B. Ayres, **1**, 116.
PENNA (KRAUS & PENNA), 1916, *Conf. Soc. Sud. Amer. hig.*, Buenos Ayres, p. 167.
PETERMANN, M., 1892, *Ann. Inst. Pasteur*, **6**, 32.
PFEILER, W. & DRESCHER, L., 1913, *Z. InfektKr.*, **13**, 391.
PLOTZ, H., 1924, *Ann. Inst. Pasteur*, **38**, 169.
PODVISOTSKI, V. V. & TARANUKHIN, V. A., 1898, *Russk. Arch. Patol., Klin. Med. i Bakt.*, S. Peterb., cited from *Zbl. Bakt.*, **5**, 653.
POHL, G., 1927, *Zbl. Bakt.*, Abt. I, Orig., **103**, 368, 371.
PONDER, C. W., 1911, *Report to Worshipful Co. of Leathersellers on the Incidence of Anthrax amongst those engaged in the Hide, Skin and Leather Industries.* . . . Published by the Company and *Lancet*, Lond., ii, 1260.
PRAZMOWSKI, A., 1885, *Biol. Zbl.*, **4**, 393.
RAYER, P., 1850, *C.R. Soc. Biol.*, Paris, **2**, 141.

Reichel, 1911, *Zbl. Bakt.*, Abt. I, Ref., **50,** Beilage 83.*

Reiter, R., 1920, *Arch. Hyg.*, Berl., **89,** 191.

Renshaw, A., personal communication (re Auramin).

Rosatzin, T., 1899, Lubarsch *Zur Lehre von den Geschwülsten und Infektionskrankheiten,* Wiesbaden, 77.

Roux, 1921, cited by Besson, in *Technique microbiologique.*

Sabolotnyï, S. S., 1926, *Zbl. Bakt.*, Abt. I, Orig., **99,** 53.

Sachelarie, V., 1925, *Rev. gén. Méd. vét.*, **34,** 229.

Sanarelli, G., 1924, *C.R. Acad. Sci.*, Paris, **179,** 937, 1100; 1925, *Ann. Inst. Pasteur,* **39,** 209.

Sanfelice, F., 1924, *Boll. Ist. sieroter.*, Milano, cited from 1925, *Zbl. Bakt.*, Abt. I, Ref., **79,** 436.

Schütz & Pfeiler, 1911–12, *Arch. wiss. prakt. Tierhlk.*, **38,** 207, 311; 1913, *ibid.*, **40,** 395; 1914–15, *ibid.*, **41,** 286.

Sclavo, A., 1895, *Zbl. Bakt.*, Abt. I, **18,** 744.

Senge, J., 1913, *Zbl. Bakt.*, Abt. I, Orig., **70,** 353.

Silberschmidt, W. & Schoch, E., 1920, *Ann. Inst. Pasteur,* **34,** 669.

Sobernheim, G., 1897^1, *Berl. klin. Wschr.*, **34,** 910; 1897^2, *Z. Hyg. InfektKr.*, **25,** 301; 1899, *Berl. Klin. Wschr.*, **36,** 273.

Standfuss, R. & Schnauder, F., 1925, *Zbl. Bakt.*, Abt. I, Orig., **95,** 61.

Szász, A., 1918, *Z. InfektKr.*, **19,** 367.

Szymanowski, Z. & Zagaja, J., 1911, *Z. InfektKr.*, **12,** 256.

Tada, S., 1924, *Zbl. Bakt.*, Abt. I, Orig., **91,** 477.

Tatin & Velu, 1926, *C.R. Soc. Biol.*, Paris, **94,** 334.

Turró, R., 1919, *C.R. Soc. Biol.*, Paris, **82,** 1085.

Uschida, Y., 1926, *Z. Hyg. InfektKr.*, **106,** 275.

Velu, H., 1924, *C.R. Soc. Biol.*, Paris, **90,** 746; 1927, *Ann. Inst. Pasteur,* **41,** 615.

Wilke, H., 1919, *Untersuchungen über präzipitierende Eigenschaften des Fäzes milzbrandkranker Schweine und Rinder,* Diss., Giessen.

Wollman, E., 1925, *C.R. Soc. Biol.*, Paris, **92,** 127.

Zehetmayr, H., 1922, *Zbl. Bakt.*, Abt. I, Orig., **89,** 153.

Zironi, A., 1919, *Ann. Igi.* (*sper.*), **29,** 493.

Zwick, W., 1913, *Z. InfektKr.*, **14,** 91.

CHAPTER XI. THE NON-PATHOGENIC SPORE-BEARING AEROBIC BACTERIA.

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Classification.

The aerobic spore-bearing bacteria, other than *B. anthracis*, comprise a large group of more or less closely related organisms, the differentiation of which has always presented considerable taxonomic difficulties. They are, for the most part, saprophytic and non-pathogenic, but certain members are occasionally responsible for accidental infections in man and animals, and are, to this extent, of pathogenic importance. Typically, these bacteria are motile, Gram-positive rods, liquefying gelatin and actively decomposing protein media through the action of enzymes.

The original descriptions of these organisms were often faulty and incomplete, and unfortunately in cases where the original cultures have been lost, subsequent observers have experienced great difficulty in determining the exact species to which reference was made. For our present knowledge of the group we are indebted to the researches of Gottheil (1901), Neide (1904), Chester (1904), and more recently to Ford (1916) and his co-workers. These latter investigators acknowledged some twenty-eight species of non-pathogenic spore-bearing bacteria, which they have divided into nine groups, the salient characters of which are as follows :

Group I. Subtilis Group.

Small homogeneous sluggishly motile organisms. No threads on glucose agar. Central or eccentric spores, oval, often retaining terminal tags of protoplasm. Growth on solid media hard and penetrating, with tenacious scum on fluid media.

Represented by
B. subtilis (Ehrenberg) Cohn.
B. subtilis viscosus Chester.

Group II. Mesentericus Group.

Small homogeneous actively motile organisms. Often produce long threads on glucose agar. Spores, oval, retaining terminal tags of protoplasm. Growth on hard media as soft pultaceous mass with tendency to wrinkle, on fluid media as friable easily broken scums.

Represented by
B. vulgatus (Flügge) Trevisan (*B. mesentericus vulgatus* Flügge).
B. mesentericus (Flügge) Migula (*B. mesentericus fuscus* Flügge).

B. aterrimus Lehmann and Neumann (*B. mesentericus niger* Lunt).
B. globigii Migula (*B. mesentericus ruber* Globig).
B. niger Migula (*B. lactis niger* Gorini).
B. mesentericus var. *flavus* Laubach.
B. panis Migula (*B. mesentericus panis viscosi I* Vogel). (Motility lost by capsule formation.)

(The above groups are now included by Ford [1927] in one group—the subtilis-mesentericus group.)

Group III. Cohærens-simplex Group.

Motile organisms somewhat larger than the preceding. Thicker and longer forms on glucose agar. Involution and shadow forms common and appear early. Spores cylindrical. Growth as soft mass on hard media, as turbidity with little or no scum on fluid media.

Represented by
B. cohærens Gottheil.
B. simplex Gottheil.
B. agri Laubach and Rice.

Group IV. Mycoides Group.

Large organisms with square ends growing in long chains. On glucose agar organisms are thicker and longer and made up of globular bodies. Tendency to grow in curves or spirals. Spores central or eccentric, round or oval to cylindrical. Spores vary greatly in size and often appear in chains. Growth on hard media dry and penetrating, on fluid media as firm tenacious scum.

Represented by
B. mycoides Flügge.
B. prausnitzii Trevisan (*B. ramosus liquefaciens* Prausnitz).
B. adhærens Laubach (no motility).

Group V. Cereus Group.

Large motile organisms with round ends. Tend to grow in short chains. Thicker and longer on glucose agar where protoplasm is converted into globular bodies. Central or eccentric cylindrical spores. Spores retain protoplasm at one or both ends, often resembling enlarged *subtilis* or *mesentericus* spores. Growth on hard media as soft pultaceous mass with tendency to fold or wrinkle, on fluid media as thick friable scum.

Represented by
B. cereus Frankland.
B. cereus var. *fluorescens* Laubach.
B. albolactis Migula.

Group VI. Megatherium Group.

Very large actively motile organisms. Often in long forms which spread out, lose their cytoplasm and show peculiar aggregations of protoplasm at their periphery. Protoplasm rapidly converted into peculiar globular highly refractile bodies, particularly on glucose agar. Shadow and transparent forms appear early. Spores central, eccentric or subterminal oval to cylindrical. Spores vary greatly in shape, sometimes round, sometimes rectangular, often reniform. Growth on solid media as thick pultaceous mass, on fluid media as turbidity with little or no scum formation.

Represented by
B. megatherium De Bary.
B. petasites Gottheil.
B. ruminatus Gottheil.

Group VII. Round Terminal-Spored Group.

Small actively motile organisms, often forming long threads in old cultures. Protoplasm homogeneous. Spores subterminal or terminal, round, thicker than the organisms from which they spring.

Represented by
B. pseudotetanicus (Krause) Migula (*B. pseudotetanicus* var. *aerobius* Krause).
B. fusiformis Gottheil.

Group VIII. Cylindrical Terminal-Spored Group.

Small thin actively motile organisms. Slightly larger on glucose agar but no change in character of protoplasm. Spores terminal, cylindrical.

Represented by
B. circulans Jordan.
B. brevis Migula.
B. terminalis Migula.

Group IX. Central-Spored Group.

Long actively motile organisms with pointed ends. Slightly larger on glucose agar, but no change in character of protoplasm. Spores develop in the middle of the rods, which become spindle-shaped. Spores large cylindrical.

Represented by
B. centrosporus Ford.
B. laterosporus Laubach.

Three of these groups are of occasional pathogenic significance: the *mycoides* group, in which may conveniently be placed the various motile organisms exhibiting branching cultures which have been described as *B. pseudoanthracis*, the *cereus* group which according to Ford (1927) comprises many organisms which have previously been described as *B. anthracoides*, and the *subtilis-mesentericus* group which includes organisms resembling or identical with *B. subtilis* or *B. mesentericus*, which have been met with in ophthalmic and other infections.

The pathogenic members of the two first groups mentioned comprise such organisms as have, from time to time, been described as Pseudo-anthrax Bacilli.

Pseudoanthrax Bacilli.

From the point of view of differentiation from true *B. anthracis* it is convenient to consider together the various strains that have been described as Pseudoanthrax Bacilli, although as above stated they fall in two distinct taxonomic groups.

Causal organisms. McFarland (1898) described an organism under the name of *B. anthracis similis* with cultural characters very similar to *B. anthracis*, non-pathogenic to guinea-pigs, rabbits and white mice, but differing from previously recognized strains of *B. pseudoanthracis* and *B. anthracoides*. Bainbridge (1903) isolated an anthrax-like organism from Chinese hair. In morphology and growth in broth and on agar it resembled *B. anthracis* with regard to size, shape and capsule-staining reaction, but it was distinguished by its motility and capacity for rapidly liquefying gelatin. At no time could lateral outgrowths into the surrounding medium be observed. The organism was pathogenic to mice, on subcutaneous inoculation, causing acute septicæmia, but was not pathogenic for guinea-pigs.

Wilamowski (1912) described a case of human infection with acute inflammation of the lungs, in which motile Gram-positive and in other respects anthrax-like organisms were isolated from the pleural exudate, spleen, liver and bone-marrow. The bacilli, which were feebly motile, were pathogenic for mice and guinea-pigs, causing a rapidly fatal septicæmia in the former.

Neufeld (1913) also isolated a pseudoanthrax organism from the blood of a man with clinical symptoms of anthrax.

Grierson (1928) has recently isolated from shaving-brushes a series of organisms which he describes under the name of *B. anthracoides*, closely resembling the organism of Bainbridge above described. These organisms were pathogenic to mice and guinea-pigs in large doses, but the virulence could not be exalted. In contradistinction to *B. anthracis*, small doses of the virus were not lethal.

Morphology. The morphological resemblance between *B. anthracis* and pseudoanthrax bacilli is often so great that microscopical examination is unreliable as a means of differentiation.

Motility. Pseudoanthrax bacilli in young cultures are for the most part motile, but after the beginning of spore formation they generally become non-motile and so may be readily confused with *B. anthracis*.

Capsule formation. The pseudoanthrax bacilli, as distinguished from *B. anthracis*, do not produce a capsule which can be differentiated by staining.

Spore production. The spore production of the pseudoanthrax bacilli is more abundant and energetic than with anthrax bacilli and in agar cultures is generally completed within 24 hours. The spores are larger and more rounded than is the case with anthrax bacilli.

Cultural characters ; Surface colonies. The surface colonies of the pseudoanthrax bacilli on agar plates have, in comparison with anthrax bacilli, a less curl-like construction, and have fewer but more irregular marginal ramifications. Accordingly, in impression preparations, the pseudoanthrax bacilli show irregular threads breaking up into single bacilli. Anthrax bacilli, on the contrary, exhibit irregular parallel bacillary threads.

Growth in broth. The pseudoanthrax bacilli produce a denser initial growth in broth than *B. anthracis*, and generally form a surface pellicle which falls to the bottom of the tube on shaking. The cultures clear themselves in time, producing a definite precipitate that is differentiated from that of *B. anthracis* by its denser character.

Fermentation reactions. Anthrax, as well as pseudoanthrax bacilli, produce acid in glucose broth, while in mannitol and lactose alteration of the reaction does not occur. The pseudoanthrax bacilli generate alkali in litmus milk, through which the media becomes obviously blued. Anthrax bacilli, on the other hand, either leave the reaction unaltered, or, as may be observed with the majority of strains, produce acid with reddening of the media.

Growth on potato. Organisms belonging to the *B. pseudoanthracis* group form a reddish-brown pigment in potato culture, while those of the *B. anthracoides* group produce a growth of greyish-brown colour. Cultures of *B. anthracis* are, on the other hand, of a yellowish coloration.

Gelatin. In gelatin stab cultures the pseudoanthrax bacilli also show two typical individual growth forms. *B. pseudoanthracis* group organisms produce luxuriant extensive growth at right angles to the inoculation stab in the form of self-ramifying branches. They are thus separated from true anthrax bacilli, in which the lateral branches are fewer and closer together, so that the culture has the appearance of a lamp brush. The other group of pseudoanthrax bacilli (*B. anthracoides*) are distinguished by the button-like nodular colonies which appear along the sides of the stab canal, and do not extend into the breadth of the media. Both these groups of organisms liquefy gelatin more rapidly than *B. anthracis*.

In the *B. pseudoanthracis* group the liquefaction proceeds parallel to the surface; in the *B. anthracoides* group it broadens out along the stab canal, so that the lower part of the liquefaction zone has a funnel-shaped appearance. Growth in agar stab is similar, except, of course, that there is no liquefaction.

Precipitation reaction. With the precipitation method of Ascoli (1911) precipitating anthrax serum gives a positive reaction with pseudoanthrax extract as well as with anthrax extract. The precipitation is, therefore, a group reaction and the differences observed are purely of a quantitative nature. For example, an extract of anthrax culture may react with a precipitating anthrax serum up to an extract dilution of 1 in 50, while extracts from pseudoanthrax cultures give a positive reaction up to a dilution of, say, 1 in 20. On the other hand, a pseudoanthrax serum which reacts with pseudoanthrax extracts up to a dilution of 1 in 50 may only react with anthrax extracts up to a dilution of about 1 in 10. According to Pfeiler and Drescher (1913), however, the distinction in this regard is not sufficiently great to be of real differential value and is not of practical application. Pohl (1927) has recently shown that pseudoanthrax organisms on dried imported hides do not give positive reactions with precipitating anthrax serum, and that a positive Ascoli reaction in such cases is pathognomonic of anthrax.

Complement fixation. The complement-fixation test is also of value as a group reaction, even if not in so marked a grade as with precipitation.

Hæmolysis. Anthrax and pseudoanthrax bacilli may also be distinguished by their growth on blood media. Anthrax colonies do not produce any hæmolytic effect, while pseudoanthrax cultures develop a varying degree of hæmolysis.*

Taxonomic grouping. On account of the growth peculiarities of pseudoanthrax bacilli on agar and gelatin, Pokschischewsky (1914) has separated these organisms into two groups:

1. *Typus pseudoanthracis* (including *B. pseudoanthracis* Wahrlich [1892]), which exhibit in gelatin and in agar stab cultures luxuriant self-ramifying branches and give a reddish-brown pigment on potato; and
2. *Typus anthracoides* (including *B. anthracoides* Hueppe and Wood [1889]), which form button-like colonies in gelatin and agar and produce a dirty grey growth on potato.

The Subtilis-Mesentericus Group.

Pathogenicity. Organisms of the *subtilis-mesentericus* group are common in soil, dust, water and milk, and are frequently met with in the dejecta of man and animals. They are generally described as non-pathogenic, but under certain conditions are able to take on pathogenicity, and may cause infections, especially of the eye.

Silberschmidt (1903) described a case of human panophthalmia caused by *B. subtilis.* His description of the organism, however, does not agree with that of Chester (1904) or of Ford (1927). Seitz (1913) described a pathogenic *B. subtilis* isolated in pure culture from the slimy evacuations of a sick Chinese. Morphologically and culturally the organism resembled *B. anthracis*, but the feeble motility and the granular form of the colonies affirmed the diagnosis of *B. subtilis.* Senge (1913) found feebly motile anthrax-like rods in the lumbar fluid as well as in the brain and spinal cord in a case of meningitis purulenta and encephalitis hæmorrhagica. The organism, which was fatal to mice and guinea-pigs, resembled *B. subtilis* in agar cultures and appeared to belong to the *subtilis-mesentericus* group.

Rosenow and Sandford (1915) described an organism of the *subtilis* group as ancillary to streptococcic infection found in excised gastric ulcers. Lindberg (1916) describes a fatal case of meningitis in an infant in which a typical *B. subtilis* was isolated from the meningeal exudate and from the heart-blood.

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* According to Hallermann (1925), however, *B. anthracis* may give slight hæmolysis on 5 per cent. blood-agar plates, which can be increased by growth in alcohol broth.

v. Kleczkowski (1917) obtained an organism, stated to resemble *B. mesentericus*, from an abscess following injury to the conjunctiva, which produced iritis and infection of the vitreous in rabbits. Reitsch (1922) reported a case of *subtilis* panophthalmia following non-perforating injury to the eyeball, the organism being obtained from pus present in the vitreous body. The infection was considered as secondary to a suppurating wisdom tooth from which a culture of the same organism was also obtained.

Fiori (1924) isolated from a fatal case of acute peritonitis an organism like *B. subtilis*, which after passage through guinea-pigs showed strong affinities to the anthrax bacillus. Sweany and Pinner (1925) described a pathogenic *B. subtilis* isolated from the heart-blood, serous cavities and abscesses of a patient with chronic tuberculosis. The organism, which was pathogenic for white rats, could at first only be grown on blood media, on which it produced a broad hæmolytic zone. Later, however, it was cultivated on ordinary media, on which the pathogenicity rapidly disappeared.

Taxonomy. A great deal of confusion exists with regard to the classification and nomenclature of this group of organisms. It is difficult to be certain, for example, as to the exact organism originally described by Ehrenberg and Cohn as *B. subtilis*, or if any of the pathogenic members of the group cited in the literature correspond to the original isolation of Ehrenberg. The designation '*Bacillus subtilis*' has often been given loosely to various members of the group.

It would seem necessary, therefore, to append a brief account of *B. subtilis* and *B. vulgatus*—the two most important representatives of the *subtilis-mesentericus* group. The descriptions of Ford and his co-workers are followed.

BACILLUS SUBTILIS (EHRENBERG) COHN.

This organism, known as the 'Hay Bacillus', has often been confused with *B. cereus* Frankland, but in all probability the description given by Gottheil, Chester, and more recently by Ford and his co-workers, is the correct one, and corresponds with that of the original isolation of Ehrenberg.

Morphology. Small, thin, homogeneous Gram-positive bacilli, measuring 0·4μ by 1·5 to 2·5μ in 24-hour agar cultures. Do not form threads on glucose agar. Spores appear early at centre or towards one end of the rods. They are wider than the vegetative rods, in which they cause bulging. Free spores retain pieces of protoplasm at each end.

Motility. Sluggish motility in young broth cultures. Peritrichous flagella present.

Cultural characters. The bacillus grows readily and luxuriantly on all media. Plate cultures and agar slants exhibit surface colonies with spreading membranous, hard, dry and glassy growth, which can only be separated from the agar with difficulty. In agar stab culture there is little growth along the line of inoculation, but a spreading dry membranous growth extends to the wall of the test-tube. In gelatin stab culture there is a slow growth along the line of inoculation, and a rather slow surface cup-shaped liquefaction with scum production. There is luxuriant and warty dew-drop growth on potato, on which pinkish pigment is developed in 48 hours. Old cultures become moist and sticky. Litmus milk is made alkaline with no coagulation. Growth in broth is characteristic—a thin branching scum is formed in 48 hours, which later becomes dense and tough and is precipitated in about 10 days. Capsules are not formed and blood media are not hæmolysed.

BACILLUS VULGATUS (FLÜGGE) TREVISAN
(*B. MESENTERICUS VULGATUS* FLÜGGE).

This organism, known as the ' Potato Bacillus ', is almost as common as *B. subtilis*, with which it has often been confused.

Morphology. Small homogeneous organisms usually distinctly larger than *B. subtilis*, measuring $0 \cdot 5\mu$ by $2 \cdot 0$ to $3 \cdot 0\mu$. There is no tendency to form threads on glucose agar. Spores are similar to those of *B. subtilis*, but do not appreciably bulge the vegetative rods, being about the same width.

Motility. Active, progressive and rotatory motility in young cultures. Flagella peritrichous.

Cultural characters. Surface colonies on agar plates are round and waxy, with edges entire. In agar stab culture there is little growth along the line of inoculation, but a dry wrinkled rooty growth spreads along the surface of the agar. In gelatin stab culture cup-shaped and surface liquefaction is observed with scum production. The growth on potato is characteristic, thick white, grey or pink folds are formed within 24 hours, and later become a brown reticulated mass. Milk is peptonized but not coagulated or acidified. There is turbidity and scum production in broth in 24 hours : the medium gradually clears itself. There is no capsule formation or hæmolysis of blood media.

REFERENCES.

ASCOLI, A., 1911, *Deuts. med. Wschr.*, **37**, 353.

BAINBRIDGE, F. A., 1903, *J. Path. Bact.*, **8**, 117.

CHESTER, F. D., 1904, *Zbl. Bakt.*, Abt. II, **13**, 737.

FIORI, A., 1924, *Bollet. Ist. Sieroter.*, Milano, **3**, No. 2, cited from *Zbl. Bakt.*, Abt. I, Ref., **77**, 435.

FORD, W. W., 1927, *Text Book of Bacteriology*, W. Saunders Co., 1069 pp.

GOTTHEIL, O., 1901, *Zbl. Bakt.*, Abt. II, **7**, 430.

GRIERSON, A. M. M., 1928, *J. Hyg.*, Camb., **27**, 306.

HALLERMANN, A., 1925, *Zbl. Bakt.*, Abt. I, Orig., **96**, 419.

HUEPPE, F. & WOOD, C., 1889, *Berl. klin. Wschr.*, **16**, 347.

v. KLECZKOWSKI, T., 1917, *Arch. Augenhlk.*, **82**, 26, cited from reference in *Zbl. Bakt.*, Abt. I, Ref., 1919, **68**, 197.

LAUBACH, C. A., RICE, J. L. & FORD, W. W., 1916, *J. Bact.* **1**, 493.

LINDBERG, G., 1916, *Zbl. Bakt.*, Abt. I, Orig., **78**, 302.

MCFARLAND, J., 1898, *Zbl. Bakt.*, Abt. I, **24**, 556.

NEIDE, E., 1904, *Zbl. Bakt.*, Abt. II, **12**, 1.

NEUFELD, 1913, *Bericht über die 7 Tagung der Freien Vereinigung für Microbiologie in Berlin, Zbl. Bakt.*, Abt. I, Ref., **57**, 279.*

Pfeiler, W. & Drescher, L., 1913, *Z. InfektKr.*, **13**, 391.
Pohl, G., 1927, *Zbl. Bakt.*, Abt. I, Orig., **103**, 371.
Pokschischewsky, N., 1914, *Arb. GesundhAmt.*, Berl., **47**, 541.
Reitsch, W., 1922, *Klin. Mbl. Augenhlk.*, **69**, 100.
Rosenow, E. C. & Sanford, A. H., 1915, *J. Infect. Dis.*, **17**, 219.
Seitz, A., 1913, *Zbl. Bakt.*, Abt. I, Orig., **70**, 113.
Senge, J., 1913, *Zbl. Bakt.*, Abt. I, Orig., **70**, 353.
Silberschmidt, W., 1903, *Ann. Inst. Pasteur*, **17**, 268.
Sweany, H. C. & Pinner, M., 1925, *J. Infect. Dis.*, **37**, 340.
Wahrlich, W., Bakteriologische Studien (S.A., aus Scripta Botanica, p. 30, mit 3 Tafeln, St. Petersburg, 1890–91), cited from *Zbl. Bakt.*, 1892, **11**, 49.
Wilamowski, B. I., 1912, *Zbl. Bakt.*, Abt. I, Orig., **66**, 39.

CHAPTER XII. TULARÆMIA (SYNS. DEERFLY FEVER, RABBIT FEVER, OHARA'S DISEASE) AND ITS CAUSATIVE ORGANISM, *B. TULARENSE.*

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Historical Introduction.

Tularæmia is a disease of rodents communicable to man either directly, or indirectly through the medium of insect vectors, and confined, so far as is known, to the United States of America and Japan.* Its history as a disease of rodents dates from 1911, when McCoy in the course of an enquiry into the presence of plague infection among the ground squirrels of California encountered in certain autopsied animals lesions of remarkably plague-like character, which failed to yield cultures of *B. pestis.* He described the new disease as a 'plague-like disease of rodents'. In the same year he and Chapin succeeded in isolating from the lesions in affected ground squirrels an organism named by them *B. tularense,* so called from Tulare County in California, where the rodent disease was particularly rife.

There is no definite record of human cases of infection having arisen in connection with the ground-squirrel disease, but it is significant, in the light of subsequent discovery, that, in the course of their dissection of squirrels, Chapin suffered from a febrile illness which lasted 28 days, and both his serum and that of a laboratory attendant were found to contain specific antibodies for *B. tularense.* The history of tularæmia as a clinical disease entity in man really dates from observations made in the State of Utah, since 1908 or earlier, on cases of what was locally known as deerfly fever following the bite of a fly. At the site of the bite inflammatory processes with ulceration and sloughing occurred followed by involvement of neighbouring glands. It was not, however, till 1919 that Francis established the fact that these cases of deerfly fever were due to infection by *B. tularense,* conveyed to man from jack rabbits by the bite of *Chrysops discalis.* These jack rabbits and other varieties of rabbit (snowshoe rabbits, cotton-tail rabbits, &c.) have since proved to be a most potent source of human infection in many states of the Union. The first demonstration of *B. tularense* in man was made by Wherry and Lamb (1914) in connection with three cases of ulcerative conjunctivitis accompanied by fever and glandular enlargement which occurred in the practice of three ophthalmic surgeons of Cincinnati (Ohio).

In 1923 Francis reported the finding of *B. tularense* in infected rabbits exposed for sale in the Washington market and elicited the information that a disease known to the trade as rabbit fever was not infrequent among rabbit skinners and market workers.

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*A tularæmia focus has now been definitely recognized in Siberia (Obdorsk District) (Sarchi, 1929).

In 1924 Parker, Spencer and Francis demonstrated *B. tularense* infection in wood-ticks (*Dermacentor andersoni*) in the state of Montana. These ticks apparently determine the continuance of an epizootic among rodents and they may possibly convey infection to man, though clinical data on this point are meagre. In 1922 Lake and Francis reported the occurrence of tularæmia with typhoid-like syndrome among workers investigating tularæmia in the laboratory. Under similar conditions three laboratory cases of infection occurred in the Lister Institute, London (Ledingham and Fraser, 1923), and additional cases have since been reported at the Rocky Mountain Spotted Fever and Tularæmia Laboratory at Hamilton, Montana, by Parker and Spencer (1926[1]), and at the Los Angeles Plague Laboratory, by Dieter (1926), himself the victim.

In 1925 Ohara drew attention to a clinical disease entity occurring in the Abukuma Mountains in the eastern part of Fukushima prefecture, and due, in his opinion, to handling the uncooked meat of wild rabbits which at certain seasons were wont to succumb in great numbers. That infection might be so conveyed to man was proved experimentally by Madame Ohara's allowing herself to be inoculated by inunction of the skin of the hand with presumably infective material from a rabbit found dead in the district where these human cases had occurred. A typical attack followed (see later). That the human cases were really due to infection by *B. tularense* was proved by results obtained at Washington with sera from human cases transmitted from Japan and with an excised human lymph gland which yielded a culture of *B. tularense* after guinea-pig passage in Washington (Francis and Moore, 1926). In concluding this historical sketch of the main landmarks in tularæmia research since 1911, when the disease was first brought to notice by McCoy, it seems but fitting to acknowledge the debt of bacteriologists to the fruitful researches of Francis and his co-workers at the Washington Public Health Bureau. The great part of our present knowledge of this interesting disease we owe to a succession of illuminating researches on tularæmia problems which have emanated from that laboratory.

Mechanism of Spread of Infection in Nature from Rodent to Rodent and from Rodent to Man.

In the so-called deerfly fever as observed in Utah, infection is conveyed to man by the bite of *Chrysops discalis* which has previously fed on an infected jack rabbit. The victims are usually farmers and the infective bite, generally about the temple or aural region, is followed by inflammatory and suppurative adenitis. The human disease is most frequent in Utah during the seasonal prevalence of this fly—June to August. The presence of the disease among the jack rabbits was settled by Francis in 1920, when, of 556 shot rabbits, 23 presented suspicious lesions. In 17 of these 23 the infection was proved by guinea-pig passage. In 1923 (Francis, 1923[1]), when Francis discovered 7 infected rabbits exposed for sale in the Washington market, he learnt that the owner of the stall was in the habit of removing the viscera with his bare hands and that his immunity was in all probability due to his having, 12 months previously, suffered from a febrile illness of a month's duration with enlarged axillary glands. The three cases in Cincinnati in which the conjunctiva was the primary seat, and which were the first human cases to be definitely proved by recovery of the organism (Wherry and Lamb, 1914), also acquired infection from rabbits. One, we are told, was a meat-cutter in a cheap restaurant and the other two had prepared rabbits for the family dinner. Infection was almost certainly conveyed by rubbing an infected finger over the eye. Freese, Lake and Francis (1926) record four simultaneous cases of this type in one family, three of which proved fatal in 6 to 8 days. The onset in this unusually serious group of cases was sudden, with high fever and nausea followed by enlargement of glands in parotid, cervical and axillary regions, and severe conjunctivitis. The presence of *B. tularense* in throat and nasal washings of the survivor, a girl of six years, was proved by guinea-pig passage, and her serum was found to agglutinate the organism in a dilution of 1 in 1,280. The reporters of these cases subsequently showed that rabbits could be readily infected by dropping minute amounts of virulent culture into their conjunctivæ, and that death took place preceded by severe conjunctivitis with enlargement and caseation of regional glands. Also they demonstrated the presence of viable organisms capable of infecting guinea-pigs in the inner portions of infected rabbit limbs which had been insufficiently cooked.

The importance of wood-ticks (Dermacentor andersoni). In 1924, while examining wood-ticks for Rocky Mountain Fever virus by injecting them into guinea-pigs, Parker, Spencer and Francis occasionally observed lesions suggestive of tularæmia. *B. tularense* was readily recovered from these lesions. Following these observations a series of experiments was undertaken to determine the presence of infection in the various developmental stages of the tick (eggs, larvæ and nymphs). Larvæ of *D. andersoni* were fed on infected guinea-pigs and reared to the adult stage, and both nymphs and adults were found to be infective when tested subsequently on guinea-pigs. Later, Parker and Spencer (1926[2]) amplified this work by demonstrating experimentally that the infection of the adult wood-tick could be transmitted to the egg. In 8 of 15 parent females fed on infected hosts, *tularense* infection was found to be transmitted to eggs, larvæ, and nymphs. These experiments certainly throw light on at least one mechanism by which *tularense* infection may be maintained in nature among rodent populations, and doubtless this conservation of infection is further assured by the fact of hereditary transmission. It is not quite certain whether man may acquire infection from tick bites, but investigation of certain illnesses following tick bites is, we understand, in progress.

Natural infection of wild animals other than jack rabbits and ground squirrels. Coyote (Canis lestes). Parker and Francis (1926) fed three coyotes on tissues of infected hares and guinea-pigs, and all three died, in 53, 22 and 13 days respectively, after the infective feed. There were no visceral lesions at autopsy, but *B. tularense* was proved to be present in the carcasses by guinea-pig passage, and in two of them in the salivary glands. These experiments were undertaken in view of the occurrence of a human case which developed infection after the bite of a coyote. Apparently, therefore, the coyote may not simply have transferred infection mechanically to man after a meal of infected rabbit.

Wood-mice (Microtus californicus æstuarinus). Perry (1928) has demonstrated the presence of tularæmia infection among these migrating rodents in Contra Costa County (Cal.). Two dead rodents which were dissected presented no gross pathological lesions. Infection is probably kept alive among them by mites.

Mechanism of infection in laboratory workers. Eighteen such cases have now been reported from various laboratories in which tularæmia has been under experimental investigation. In no case, with one possible exception, that of Dieter (1926), have there been local lesions indicating a portal of entry, and as a rule the utmost precautions have been taken to prevent infection while handling experimental animals. It is known, however, that *B. tularense* may penetrate the unbroken skin, and that the conjunctiva is possibly a vulnerable spot. A droplet respiratory infection was suggested by Ledingham and Fraser (1924) in the case of one of the victims at the Lister Institute. The onset in his case took place nine days after a single exposure while inducing anæsthesia in an infected guinea-pig, preliminary to passage of the virus. In the early stage of induction the animal sneezed in his immediate presence. This was his sole association in any form with tularæmia. In a discussion of possible sources of infection in the six cases at the Montana laboratory reported by Parker and Spencer (1926^1) it is of considerable interest that the authors draw attention to one incident that may have had a bearing on the mode of infection in three of the victims concerned. Engorged female ticks were being removed from a rabbit on May 16, and during the process the rabbit began a loud and prolonged squealing. One worker held the rabbit, a second removed the ticks, while the third, attracted by the squealing, came and leant over the rabbit and the table. All three became ill within 48 hours of this incident. The rabbit succumbed to tularæmia eight days after removal of the ticks and autopsy revealed marked involvement of the lungs.

Experimental Transmission of Tularæmia.

Susceptibility of various animals to experimental infection. Laboratory mice, guinea-pigs and rabbits are extremely susceptible, and in mice particularly the infection is most intense. Rats, on the other hand, both wild and white rats, display considerable resistance. From observations by Dieter and Rhodes (1926) in California it would appear that wild rats examined in the course of plague enquiries may occasionally be found to harbour *tularense* infection in a latent form demonstrable only by transference of rat tissues to the guinea-pig. The authors inoculated a series of rats with a constant dose of a virulent culture, the result being that 65 per cent. succumbed to infection. The surviving rats were killed at intervals varying from 10 to 21 days. None showed suggestive lesions, but the persistence of *B. tularense* in their spleens and livers was determined by guinea-pig passage. They also elicited the interesting fact that rats succumb to intraperitoneal inoculation of *B. tularense*, so that the route of infection is of importance in estimating the apparent resistance of these animals. The desert ground squirrel (*Ammospermophilus leucurus*) and the ground squirrel (*Citellus beecheyi**) are readily infected. According to McCoy and Chapin (1912), calf, swine, and goat are quite resistant, but experiments on sheep† revealed some degree of susceptibility. Cats and dogs they also found to be resistant, but the question of the cat's susceptibility has been re-investigated by Green and Wade (1928[2]) in view of some evidence showing that cats fed on rabbit that had given rise to a human case of tularæmia, sickened for a week, while another human case apparently followed the bite of a sick cat which died a few hours after inflicting the wound. Of five cats fed on infected guinea-pig carcasses, one showed mild transient symptoms, three had a serious illness lasting a week, while the fifth died on the sixth day. From the organs of the latter, *B. tularense* was recovered by guinea-pig passage. The cat, therefore, like the coyote, may acquire an active infection. With regard to birds, the simultaneous diminution of wild rabbits and grouse in Montana during recent years and the occurrence of human cases have drawn attention to the possible susceptibility of grouse. Parker and Spencer (1925–6) produced an infection in blue grouse, and demonstrated the presence of the rabbit-tick (*Hæmaphysalis leporis-palustris*) on game birds. More recently, Green and Wade (1928[1 & 3]) have demonstrated the high susceptibility of the ruffed grouse (*Bonasa umbellus*) and the Hungarian partridge to experimental infection. In these birds the disease takes the form of a septicæmia. There are no macroscopic lesions in the organs. The domestic chicken, the ringnecked pheasant and the pigeon are quite resistant.

Experimental transmission by various insect vectors. An extensive series of experiments by Francis and Lake (1921), Francis and Mayne (1921) and Francis and Lake (1922[1 & 2]) determined the potentialities as transmitting agents, of various insects harboured by susceptible rodents. Success was readily achieved with the biting fly *Chrysops discalis*, the rabbit-louse *Hæmodipsus ventricosus*, the bed-bug *Cimex lectuarius*, the mouse-louse *Polyplax serratus* and the mite *Liponyssus isabellinus*. The multiplicity of potential vectors, thus revealed experimentally, would explain the conservation of infection in rodent populations in nature.

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* Now regarded as a subspecies of *Otospermophilus grammurus*.

† There has recently been reported from Montana the occurrence of tularæmia infection in dead and dying sheep and in ticks removed from their bodies. The question of possible infection of man from handling the carcasses of sheep is now raised. (Parker, R. R. and Dade, J. S. [1929], *Publ. Hlth. Rep. Wash.*, **44**, 126.)

Experimental transmission to a human volunteer. On January 20, 1925, Ohara (1925) rubbed into the back of his wife's hand some heart-blood and tissue fluids from a rabbit found dead in the district where human cases in which he was interested were occurring. Two days later there was tenderness of the left axillary gland and headache. The disease ran a febrile course for nine days accompanied by joint pains, languor, numbness of extremities, constipation and great enlargement of axillary nodes, which were ultimately removed by operation.

Morphological, Cultural and Biochemical Properties of B. tularense.

Morphology in tissues. In stained smears of spleens from infected guinea-pigs and mice, *B. tularense* appears as small round or rod-shaped bodies usually in well-defined clusters. The rod form may vary in length from 0·3 to 0·7μ with a breadth of 0·2μ. Though small, they are slightly larger than *Rickettsia* bodies in trench-fever lice, which measure 0·3 to 0·5μ. In sections of spleens and livers of guinea-pigs and mice the organisms are readily stained by Giemsa's stain, eosin-methylene-blue and Twort's stain. In human tissue, whether from biopsy or autopsy material, the organism has not so far been demonstrated by staining methods. For smears, Dieter and Rhodes (1926) recommend the following stain: Methylene blue 2·5 gm., basic fuchsine 0·15 gm., carbolic acid 10 gm., absolute alcohol 20 c.cm., and water, 200 c.cm. Stain for one minute.

Morphology in culture. B. tularense is a Gram-negative, non-motile, non-sporing organism of very small dimensions, presenting coccoid and bacillary forms. The bacillary form predominates in young cultures and the coccoid in older growths. On ordinary media such as agar, broth or milk, no growth occurs. Certain adjuvants are indispensable for growth.

Cultural methods. The egg-yolk medium introduced by McCoy and Chapin in 1912 still remains a most useful medium, especially for primary isolations and for maintenance of virulence. It is thus prepared: The yolks of fresh eggs are mixed with physiological saline in the proportion of 60 to 40. Sloped tubes are heated for 30 minutes to 72° C. and again for an hour at 72 to 73° C. The somewhat soft surface of the medium makes it unsuitable for purposes requiring mass cultures for agglutination or absorption work.

Glucose-blood-cystine-agar medium (Francis). Francis (1922^2, 1923^2) made the important discovery that the addition of cystine or cysteine hydrochloride to media containing glucose and rabbit-blood greatly favoured the growth of *B. tularense,* and facilitated not only primary isolations, but successive subcultures over long periods. Of many substances tested, cystine was the only one which favoured growth on such media, and Francis is inclined to attribute its adjuvant action to the sulphur component of the amino-acid. Preparation : Meat-extract agar is first prepared containing 1 per cent. peptone, 1 per cent. agar and 0·5 per cent. salt, titrated to pH 7·3. To this mixture are added 0·1 per cent. cystine and 1 per cent. glucose, and the whole heated in the water-bath long enough to melt the agar and destroy possible contaminants in the cystine and glucose. After cooling, rabbit-blood is added in the proportion of 5 to 10 per cent.

Other media such as blood-agar, glucose blood-agar, and serum glucose-agar (Francis, 1922[1]) are all very inferior to cystine-containing media, but they may be considerably improved by the addition of a piece of sterile rabbit spleen. In media inoculated with infective tissue growth takes place in 2 to 7 days or even later, but in subsequent subcultures growth appears in 24 to 48 hours. Cultures made directly from guinea-pig or rabbit tissues give discrete colonies, but from mouse tissues in which the infection is overwhelming confluent cultures are obtained.

According to Francis, *B. tularense* ferments glucose, lævulose, mannose and glycerin without gas formation.

Systematic position of B. tularense. This is yet uncertain. Experiments so far performed reveal no cross-immunity relationships with *B. pestis.* The fact that sera of tularæmia cases and high titre *tularense* serum may agglutinate *B. abortus* and *B. melitensis* sometimes to very high values (Francis and Evans, 1926) is a point of considerable importance. There would appear, however, to be no evidence of mutual absorption.

Diagnosis in Suspected Human Cases.

According to Francis it has not been possible so far to secure cultures by direct isolation from human material. Simpson (1928), however, has reported successful direct isolations from man in two cases, using material taken from the walls of axillary abscesses. Guinea-pigs and mice should be inoculated subcutaneously or by inunction of epilated skin with any available material from glands or ulcers of skin and conjunctivæ. Blood from a human case inoculated intraperitoneally into guinea-pigs has produced infection. Where the disease assumes the typhoid form, the serum of the patient will be found to agglutinate *B. tularense* from the second week of illness onwards and the highest titre is reached about the sixth week. Agglutinins remain demonstrable in the serum for many years after recovery, though in diminished quantity. The sera of two of the Lister Institute cases infected in 1922 still showed in July, 1928, titres of 1 in 320 and 1 in 160 respectively. These cases were originally diagnosed by agglutination tests carried out with suspensions of *B. tularense* recovered from spleens of infected mice (Ledingham, 1923) at a time when cultures were not available. Such suspensions gave identical results with cultural material when subsequently tested in parallel (Ledingham and Fraser, 1924).

Francis and Evans (1926) have discovered that the sera of tularæmia cases may very frequently agglutinate *B. melitensis* and *B. abortus*, sometimes, indeed, to the same titre as *B. tularense*, though, as a rule, to a lower degree. Of 100 sera from human cases, 37 gave cross-agglutination of *B. abortus* and *B. melitensis*, the titre being in 3 instances the same for all 3 organisms. High titre *tularense* serum behaved very similarly. On the other hand, the sera of 3 out of 8 cases of undulant fever cross-agglutinated *B. tularense*. Absorption experiments revealed no mutual absorption of agglutinins. When a human serum from a suspected case of tularæmia was found to agglutinate *Brucella* species it was generally the case that the titre was highest with *B. tularense* and that the agglutination process developed the most rapidly. Confusion would be unlikely to occur in most cases where particulars of onset are available.

Pathology of Tularæmia in Animals and Man.

Natural infections in animals. Ground squirrels: From analyses by McCoy and Chapin (1912) of lesions found in squirrels, and later observations by McCoy in 1914 (cited by Francis, 1928) it would appear that buboes are the most common lesion. In 6 of 32 shot animals examined in 1914, buboes were the only manifestation of the disease. Spleens and livers showed caseous areas in 24 and 11 cases respectively, while 1 case only exhibited lung nodules. *Jack rabbits:* Francis examined 17 infected rabbits which were shot or moribund or found dead. They presented focal lesions in spleens and livers, but no caseous glands.

Experimental infections. Guinea-pigs: In these animals the disease may run an acute or subacute course according to the virulence of the material employed for infection. In the acute infection death takes place in 4 to 6 days. The livers and spleens are enlarged and beset with minute white necrotic foci. Occasionally the lungs present similar nodules. In the subacute form following inoculation with cultures which have lost virulence, death takes place in 2 weeks to 2 months. The lymph glands are greatly enlarged and large nodes present themselves in liver, spleen and lungs. *Rabbits:* These respond similarly to guinea-pigs, except that lung nodules are more common. *Mice:* These animals die in 3 to 4 days after inoculation. Lymph glands present some slight enlargement but no caseation. Liver appears normal macroscopically, while the spleen is beset with small foci.

Man. The case mortality of tularæmia in man is probably very low and has been placed at about 3 per cent. by Goodpasture and House (1928). Simpson (1928) states that there have been 23 recorded deaths among

613 authentic cases (mortality 3·7 per cent.). Verbrycke (1924) reported the first autopsy in a case in which death occurred 18 days after onset. A second case recorded by Francis (1928) was fatal 26 days after onset, and a third reported by Bruecken and Francis and Callender (1927), about five months after ' presumptive exposure to the disease '. Goodpasture and House (1928) report a fourth complete autopsy, death having taken place 15 days after onset (severe ulcero-glandular type), while Simpson (1928) reports a fifth case in which death took place after only 4 days' illness. Francis and Callender (1927) and Francis (1928) have published a collated report of pathological and histological data relating to complete or partial autopsies and occasional biopsies. In man the subacute or chronic type of disease is characteristic as contrasted with the fulminating type in experimental animals. The acute necrotic lesions met with in experimental animals are replaced in man by chronic fibrotic and granulomatous lesions centring round primary necrotic foci in various nodes and organs. Pathologists have undoubtedly mistaken these lesions for those of tubercle, particularly as Langhans giant cells are not uncommon in the granulomatous lesions, but as a rule, the absence of tubercle bacilli shown by all tests applied, the history of positive agglutination, and above all, the evidence as to source of infection, have been sufficient to negative any such conjecture.

Some histological features of tularæmia lesions. Histological details of the acute necrotic lesions met with in the nodes and organs of guinea-pigs and mice which have succumbed to the experimental infection, will be found in papers by Woolley (1915), Ledingham (1923) and Ledingham and Fraser (1924). On the general structure of these foci no comment is necessary, but there are certain striking and important differences between the guinea-pig and mouse lesions, which are reflected in the respective natural defences of these animals. In the guinea-pig spleen the vast areas of karyorrhectic debris may be frequently permeated by polynuclear cells, and in such cases *B. tularense* may not be demonstrable either by staining or culture. When polynuclear infiltration is absent, clumps of *B. tularense* are readily demonstrable in lymph spaces and in endothelial cells, and culture is successful. In the mouse spleen the massive necroses are never accompanied by polynuclear response, and in consequence, isolations from mouse spleens result in confluent cultures. The guinea-pig liver shows isolated necrotic foci with some infiltration of polynuclears, but there are no material changes in the liver columns. On the other hand, as pointed out by Councilman and Strong (1921) and Ledingham (1923), the mouse liver shows no discrete areas of necrosis but an invasion of the whole organ with *B. tularense,* large clusters of which lie in the interacinar capillaries and inside the liver cells themselves. This feature is of considerable interest. A similar invasion of the lining epithelium of the intestinal tract of infected ticks and bed-bugs has been described and figured by Francis (1928).

In human autopsies, as we have already indicated, the residual necrotic foci in glands and organs are found to be surrounded by organized walls of newly formed granulomatous tissue containing endothelioid cells and fibroblasts, and occasionally giant cells of Langhans type. These latter cells may in some cases be very numerous ; in others, however, none may be seen. They are perhaps more likely to be present in the earlier fatal cases, and according to Francis they have even been noted occasionally in guinea-pig lesions. None were found in the earliest fatal case reported by Simpson (1928). From the standpoint of comparative histology the occurrence of these Langhans cells in the reactive tissue surrounding primary necrotic foci in human lesions, recalls the precisely similar condition in the so-called ' resolving ' plague lesions found in the rat. The structure of these lesions and the occurrence in them of Langhans cells have been described and figured by Ledingham (1907). Permar and Weil (1926) draw attention to certain changes in blood-vessels in the neighbourhood of necrotic lesions in human cases. These changes take the form of swelling and proliferation of lining endothelium, leading to obliterative endarteritis.

Immunological Problems.

Information on immunological problems presented by *B. tularense* is at present scanty, and the only available data are statements by Francis in published lectures (Francis, 1925, 1926, 1927, 1928). Protocols of experiments have not yet been published.

Immunity conferred by an attack of the disease. In one case only has a second attack been reported. This was a laboratory worker in whom, 29 months after the first attack, a small papule containing *B. tularense* developed on a crack in the finger. Some enlargement of epitrochlear and axillary glands followed, but no fever or constitutional disturbance. Market men who have had one attack have not been known to have another. In highly susceptible experimental animals the question of immunity to subsequent infection does not arise, as all succumb to the first. Only one rabbit, according to Francis, survived an experimental infection. Thirty-five days later it was given a million fatal doses of a virulent culture and remained well.

Active immunization. No immunity was conferred on guinea-pigs by inoculation with virulent cultures killed by heat or formalin, and very attenuated cultures were equally ineffective. On the other hand, white rats which exhibit some considerable natural resistance, can be easily immunized against otherwise certainly fatal infection, by sublethal followed by increasing doses of virulent culture.

Passive immunity. Two sheep which were given one injection of a virulent culture died in 31 and 23 days. Blood removed from the sheep on the fifteenth day showed considerable protective power in mice, but no therapeutic power when given 24 hours after infection. Horse-serum from immunized animals was less potent in this respect than that of sheep.

Cross-immunity relationships. Guinea-pigs which have been rendered immune to *B. pestis*, or which have survived a plague infection, exhibit no immunity to *tularense* infection. It has, in fact, been impossible to immunize guinea-pigs against tularæmia. Rabbits repeatedly inoculated with *B. abortus* or *B. melitensis* with a view to the preparation of high-titre sera have not proved immune to *tularense* infection.

Preventive Measures.

These concern at present only the laboratory worker engaged in tularæmia research and cooks and market workers, who may have to handle infected rabbits. For these persons the wearing of rubber gloves is imperative. The use of a respirator and goggles might also be suggested for the laboratory worker as an additional precaution, in view of the possibility of droplet infection from living experimental animals harbouring lung lesions.

REFERENCES.

Councilman, W. T. & Strong, R. P., 1921, *Trans. Ass. Amer. Phys.*, **36**, 135.

Dieter, L. V., 1926, *Publ. Hlth. Rep.*, Wash., **41**, 1355.

Dieter, L. V. & Rhodes, B., 1926, *J. Infect. Dis.*, **38**, 541.

Francis, E., 1919, *Publ. Hlth. Rep.*, Wash., **34**, 2061; 1921, *ibid.*, **36**, 1731; 1922^1, *ibid.*, **37**, 102; 1922^2, *ibid.*, **37**, 987; 1922^3, *J. Amer. Med. Ass.*, **78**, 1015; 1923^1, *Publ. Hlth. Rep.*, Wash., **38**, 1391; 1923^2, *ibid.*, **38**, 1396; 1925, *J. Amer. Med. Ass.*, **84**, 1243; 1926, *Proc. Chicago Inst. Med.* (Hektoen Lecture), **6**, 27; 1927, *Atlantic Med. J.*, **30**, 337; 1928, *Handb. d. path. Mikroorg.*, hrsg. Kolle Kraus u. Uhlenhuth, Jena, **6**, Lfg. 16, 207.

Francis, E. & Callender, G. R., 1927, *Arch. Path. Lab. Med.*, **3**, 577.

Francis, E. & Evans, A. C., 1926, *Publ. Hlth. Rep.*, Wash., **41**, 1273.

Francis, E. & Lake, G. C., 1921, *Publ. Hlth. Rep.*, Wash., **36**, 1747; 1922^1, *ibid.*, **37**, 83; 1922^2, *ibid.*, **37**, 96.

Francis, E. & Mayne, B., 1921, *Publ. Hlth. Rep.*, Wash., **36**, 1738.

Francis, E. & Moore, D., 1926, *J. Amer. Med. Ass.*, **86**, 1329.

Freese, H. L., Lake, G. C. & Francis, E., 1926, *Publ. Hlth. Rep.*, Wash., **41**, 369.

Goodpasture, E. W. & House, S. J., 1928, *Amer. J. Path.*, **4**, 213.

Green, R. G. & Wade, E. M., 1928^1, *Proc. Soc. Exp. Biol.*, N.Y., **25**, 637; 1928^2, *ibid.*, **25**, 856; 1928^3, *ibid.*, **25**, 515.

Lake, G. C. & Francis, E., 1922, *Publ. Hlth. Rep.*, Wash., **37**, 392.

Ledingham, J. C. G., 1907, *J. Hyg.*, Camb., **7**, 359; 1923, *J. Path. Bact.*, **26**, 132.

Ledingham, J. C. G. & Fraser, F. R., 1924, *Quart. J. Med.*, **17**, 365.

McCoy, G. W., 1911, *Publ. Hlth. Bull.*, Wash., No. **43**.

McCoy, G. W. & Chapin, C. W., 1912, *Publ. Hlth. Bull.*, Wash., No. **53**; *J. Infect. Dis.*, **10**, 61.

Ohara, H., 1925, *Kinsei Igaku*, 12, No. 5 and *Jikken Iho*, 1925, Mar. 12.

Parker, R. R. & Francis, E., 1926, *Publ. Hlth Rep.*, Wash., **41**, 1407.

Parker, R. R. & Spencer, R. R., 1925–6, *6th Bienn. Rep. Montana State Board Ent.*, p. 30; 1926^1, *Publ. Hlth. Rep.*, Wash., **41**, 1341; 1926^2, *ibid.*, **41**, 1403.

Parker, R. R., Spencer, R. R. & Francis, E., 1924, *Publ. Hlth. Rep.*, Wash., **39**, 1057.

Permar, H. M. & Weil, G. C., 1926, *Amer. J. Path.*, **2**, 263.

Perry, J. C., 1928, *Publ. Hlth. Rep.*, Wash., **43**, 260.

Sarchi, G. J., 1929, *Zbl. Bakt.*, Abt. I, Orig., **114**, 55.

Simpson, W. M., 1928, *Ann. Intern. Med.*, **1**, 1007.

Verbrycke, J. R., Jr., 1924, *J. Amer. Med. Ass.*, **82**, 1577.

Wherry, W. B. & Lamb, B. H., 1914, *J. Infect. Dis.*, **15**, 331.

Woolley, P. G., 1915, *J. Infect. Dis.*, **17**, 510.

CHAPTER XIII. BARTONELLA AND ALLIED ORGANISMS.

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In 1905, Graham-Smith, when investigating the blood of English moles, found peculiar inclusions in the red cells in 10 per cent. The structure of these bodies is best seen after staining with Giemsa's solution. They are of variable length, 0·1 to 1μ by 0·1 to 0·2μ in breadth, and have irregular contours, the longer forms being definitely rod-shaped, while some of the smaller bodies are almost round. The rods are seldom straight and many are markedly curved, especially toward one end. The ends are frequently enlarged, producing a wedge- or dumb-bell-shape. They have a light blue colour, with darker areas at the extremities, and red granules probably of chromatin can be seen usually at the ends. Small forms are also seen in which no indication of chromatin is found. The number present in the individual cell varies from 1 to 50, and they sometimes occur free in the plasma. From their morphological characters, Graham-Smith concluded that they were of parasitic nature. Since that time observers in different parts of the world have found similar bodies in a variety of rodents and other small mammals (see Wenyon, 1926). In 1911 Brumpt, regarding these bodies as protozoa, gave the new genus the name *Grahamella*, the form seen in the mole (*Grahamella talpæ*) being the type species. There has been much controversy as to the real nature of these structures, and some observers (Laveran & Marullaz, 1914; Wenyon, 1926, &c.) are of the opinion that they are not organisms, but merely peculiar degenerations of the cell allied to basophile punctation. Special importance attaches to these bodies owing to the constant occurrence of somewhat similar structures, *Bartonella*, in the red blood corpuscles of human beings in the disease Oroya Fever, and also in the endothelial cells of the skin lesions in verruga peruviana. Recently, similar appearances have been found to develop in mice and rats after splenectomy and it has been concluded that they represent a latent infection which develops in these animals.

Bartonella bacilliformis.

This organism was first described by Barton in 1905 in the red corpuscles of patients suffering from Oroya Fever, a specific febrile disease which is associated frequently with an eruption of peculiar fungating bright red yaws-like tubercles on the skin and mucous membranes (verruga peruviana). The disease is endemic in some parts of South America, and it is especially found in certain high (3,000 to 12,000 feet) narrow valleys on the western slopes of the Andes mountains between

6 and 13° south latitude. The verruga eruption may be present before the fever supervenes or may not appear at all in the course of the fever. The eruption may and frequently does occur without fever.

Various theories have been held in the past as to the cause of the condition. Barton at first thought that an organism of the paratyphoid group was responsible and acid-fast organisms were described by Letulle and Nicolle. The characteristic inclusions in the red corpuscles were described and studied by Barton (1909), Gastiaburu (1909), Darling (1911) and others and were named *Bartonella bacilliformis* by Strong in 1915 and studied by him and others in their first expedition to South America. Strong and his co-workers, as a result of their investigations, concluded that Oroya Fever and verruga peruviana were separate entities, but more recently Noguchi and Battistini have apparently substantiated the original view of the identity of the two conditions by reproducing them in animals by the injection of pure cultures of *Bartonella bacilliformis*, and they believe that the severity and occurrence of these conditions depend both on the susceptibility of the individual and on the variation in virulence of the Bartonella.

The changes in the red blood corpuscles constitute the most characteristic feature of the febrile disease and resemble those of acute pernicious anæmia. The red cells fall in number often below 1,000,000 per c.mm. with the appearance of normoblasts and megaloblasts in large numbers. There is usually a leucocytosis, which in some cases is considerable. Bartonellæ are seen in the red cells, of which often 20 to 30 per cent. are infected. In fresh blood preparations, they appear as rods measuring 1·5 to 2·5μ in length, and as rounded bodies, from 0·5 to 1·0μ in diameter, but when fixed and stained they are somewhat smaller. In fresh preparations, the organisms are endowed with independent motility, which consists of slow transition by gliding in the direction of their long axis, along with slight bending. The rods are frequently somewhat curved and occur singly, end to end in pairs, or in chains of 3 to 5. When numerous they often lie parallel to one another. V-forms, probably representing dividing organisms, are frequent; Y-forms are also not uncommon. Cross forms are rare, and may be due to organisms being superimposed. Reproduction is apparently by transverse fission. The organisms are best demonstrated in dried blood films stained by Giemsa's method. The rods as a whole have a bluish tint, their ends often being more intensely coloured. Single rods sometimes show a deep red or purplish granule, which may be of the nature of chromatin and gives the appearance of a swelling at one extremity, the rest of the rod having the usual bluish tint. Sometimes the staining is deeper at the end opposite the one showing the granule. In organisms occurring in chains the deeply staining granules cause a beaded appearance. It is evident that all the organisms do not lie in the same plane within the corpuscle. In the endothelial cells of the lymphatic glands and spleen, and in the verruga eruption itself, the organisms appear as small rounded bodies with bluish cytoplasm and small granules of chromatin, these granules being either single or multiple. Some of the cells are distended with rounded masses of uniform size, each of which contains a number of minute points of chromatin, and it has been suggested that these spheres break up into a large number of minute elements, each containing a small particle of chromatin and resembling the forms seen in the red corpuscles. These appearances led Mayer, Rocha-Lima and Werner (1913) to the conclusion that the organisms were of the nature of chlamydozoa. More recent work, however, has suggested that these bodies are really examples of *Bartonella bacilliformis* which have a diminished affinity for the various stains.

Cultivation.

Bartonella organisms were cultivated by Noguchi and Battistini in 1926. They were first recovered from the citrated blood of a patient suffering from Oroya Fever, but some time later Noguchi and Hercelles (1926) were successful in isolating Bartonella in pure culture from material from human verruga nodules. According to these writers *Bartonella bacilliformis* grows well on solid or semi-solid media containing blood of the rabbit, horse or man ; in a fluid medium it never multiplies sufficiently to be readily recognized. No growth can be obtained on ordinary agar slopes or broth. Owing to their minute size and transparency the colonies are very difficult to detect on blood slopes. Cultures on Noguchi's semi-solid leptospira medium, when the number of organisms introduced is large, are readily detected by the occurrence of a greyish haze or of punctiform colonies in the top layer. After 1 to 2 weeks at 28° C., the growth may extend to about 1 cm. below the surface and resembles that of a leptospira. When the number of organisms inoculated originally is small, several very minute discrete greyish-white granules first appear, and as time goes on each colony slowly becomes surrounded by a hazy zone. The organisms grow at 37° C. as well as at 25 to 28° C., but at the lower temperature they continue to grow and survive for a very much longer time. No growth takes place under anaerobic conditions. The organisms grow best at pH 7·8. When grown on blood slants at 25° C. they are motile for many days, and are almost uniformly rod- or spindle-shaped. In the leptospira medium small cocco-bacillary forms are found, usually fairly well separated from each other. They are less motile in this medium and usually lose all motility after 1 to 2 weeks at 28° C. There is considerable pleomorphism, and many very large flagella can be seen on dark ground examination of the cultures. Young forms of the organism show a definite double contour which is gradually lost as they mature.

The organism in culture is Gram-negative, and is stained reddish violet with Giemsa's solution. With this stain, however, the contours are not sharp and the organisms are ill-defined as if degenerate. One to four unipolar terminal flagella can be demonstrated. All the forms seen in the red cells of patients with Oroya Fever are to be found also in the cultures. Strains isolated from verruga nodules are apparently morphologically and serologically identical with those from Oroya Fever patients and have similar immunizing powers (Noguchi, 1927).

Transmission.

The chief difficulty in the way of transmission experiments in the past has been the confusion with regard to the identity of Oroya Fever and verruga peruviana. The first attempt was made in 1885 by Daniel Carrion, a medical student of Lima, who submitted to inoculation with blood from a verruga nodule. In 23 days he developed what was apparently typical Oroya Fever, of which he died 16 days later. This experiment seemed to show that Oroya Fever and verruga peruviana were manifestations of the same disease, but has been criticized on the grounds that (*a*) the patient furnishing the inoculation may have been suffering from Oroya Fever also, and (*b*) Carrion himself may have died of some septic condition following the scarification. Unfortunately no accurate account of his illness is available, and there was no autopsy (Odriozola, 1896). Since that time many attempts have been made to transmit Oroya Fever from man to animals, but entirely without success. Inoculations of material from the verruga lesion, however, have been frequently followed by positive results in apes, dogs and rabbits (Mayer, Rocha-Lima, Werner, 1913; Jadassohn and Seiffert, 1910; Townsend, 1913[1], &c.). The animals developed lesions comparable to human verruga, but without fever or the characteristic blood picture of Oroya Fever. Jadassohn and Seiffert (1910) and Strong (1915) found the monkey to be the most susceptible animal, typical lesions developing when the skin over the eyebrows was scarified and the verruga material rubbed in. The incubation period varied from 10 to 20 days, and the virus could be transmitted from animal to animal in series. Strong also repeated Carrion's experiment, and inoculated two healthy adults with freshly excised portions of the verruga nodules. After 16 days a modified verruga eruption appeared. There were, however, none of the other signs of Oroya Fever, and only a slight febrile reaction occurred before the appearance of the lesions. The patients remained well and *Bartonella bacilliformis* could not be detected in the blood on repeated examination. In 1926 Noguchi and Battistini, using pure cultures of *Bartonella bacilliformis*, recovered from a case of Oroya Fever, succeeded in producing by intradermal and other methods of inoculation in *Macacus rhesus* monkeys, lesions which were apparently identical with human verruga. Their virus gained in virulence by passage and they eventually produced in the monkey a condition which closely resembled Oroya Fever, though *Bartonella bacilliformis* was not found in corresponding numbers in the red cells, often only a few cells showing the parasites. According to these later investigations, Oroya Fever and verruga peruviana are both manifestations of infection with the same organism. Their results have been confirmed by other workers (Mayer and Kikuth, 1927; Da Cunha and Muniz, 1927; Gaillard and Robles, 1928).

Mode of infection under natural conditions. The geographical distribution, history and clinical observation of Oroya Fever and verruga peruviana have been regarded as pointing to an insect as vector of the virus. On epidemiological grounds, Townsend (1913[1]) came to the conclusion that a species of *Phlebotomus* was responsible, and named it *Phlebotomus verrucarum.* His work has recently been confirmed and extended by Noguchi *et al.* (1929). They have been able to produce Bartonella infection in monkeys by the injection of an emulsion of crushed Phlebotomi from verrugous districts, and have shown that the Bartonellæ which appear in these animals are morphologically and serologically identical with human strains. In addition to the *Phlebotomus verrucarum,* they describe a new species, *Phlebotomus noguchii,* which also readily carries the disease.

Bartonella muris ratti.

Lauda (1925) and others have shown that a few days after extirpation of the spleen, rats tend to develop a severe and often fatal anæmia, which is accompanied by fever and leucocytosis. He was of opinion that in these rodents the spleen exercised a protective function, and that when it was removed a virus which had lain latent on the gastric or intestinal mucous membrane, gained entrance to the body. Normal animals did not become infected on inoculation with blood or organs of anæmic rats, but anæmia resulted in rats from which the spleen had been removed and which had not developed the anæmia or had recovered from it. These observations were confirmed by Mayer *et al.* (1926), who noted that the red cells of the affected animals contained certain small rod-shaped structures which resembled bodies previously seen (1921) in the blood of rats suffering from trypanosomiasis which had been treated with Bayer 205. They suggested the name *Bartonella muris* on account of their resemblance to *Bartonella bacilliformis.* The Bartonella infection developed regularly in the rats at Hamburg after splenectomy. These workers also failed to produce the disease in normal animals by transference of blood or organs from anæmic splenectomized rats. Later (1927[1]), they found that *Bartonella muris* could be eliminated from the blood by treatment with various arsenical compounds. Injections of blood containing Bartonella then produced anæmia in such treated animals together with a heavy Bartonella infection of the corpuscles. Normal blood failed to produce either anæmia or the appearance of Bartonella. On this evidence they concluded that *Bartonella muris* is a living organism and the causative factor in rat anæmia following splenectomy. Lauda (1927) confirmed these results in Viennese rats, though here the infection was not so general as in Hamburg, and Nauck (1927) obtained similar results in Pekin. Haan, Lauda and Sorge (1927) showed that rats from South Italy had no *Bartonella muris* in their blood after splenectomy and did not develop anæmia ; but when injected with blood or organ emulsions from

Viennese rats and then subjected to splenectomy they developed a typical Bartonella anæmia. Jaffé and Willis (1928) have stated that rats in Illinois also harbour the infection, and they found numerous Bartonella forms in the blood of young rats before splenectomy. In other parts of the world the presence of Bartonella in splenectomized rats has also been confirmed by de Faria and Cruz (1927), Noguchi (1927), Bayon (1928), Cannon, Taliaferro and Dragstedt (1928), Ford and Eliot (1928). The course and appearance of Bartonella infection, as verified by the writer's observations in splenectomized rats and mice, is as follows. *Bartonella muris* appears in the blood in the form of minute rods (0·5 to 1·0μ in length and 0·1 to 0·2μ in thickness), and small coccoid forms about 0·05 to 1μ in diameter. There is very little variation in size and shape and the long Y and V forms which characterize *Bartonella bacilliformis* are not seen. In fresh preparations examined by the dark ground method the organisms are seen to move slowly inside the red cells and extracellular forms are not uncommon. In stained preparations the organism has a reddish tint with Giemsa's stain, but cannot be demonstrated by Gram's method and only very faintly with basic fuchsine. Occasionally the dark staining granules can be seen, usually at the ends of the rods, giving them a modified dumb-bell appearance. The number of parasites varies considerably during the course of the anæmia. At first they are scanty, occurring singly in an occasional cell, and are usually short rod forms. As the infection progresses numerous reticulocytes appear in the blood and these seem specially liable to attack, often 20 to 30 parasites being found in a single cell. Frequently they seem to be attached to the surface of the cells and can be observed projecting at the periphery. If the animal survives the organisms usually disappear quite suddenly from the blood somewhere between the fifth to tenth day after splenectomy, and the animal slowly recovers.

Up to the present there is no definite evidence that *Bartonella muris* has been cultivated, though various organisms which morphologically resemble it have been isolated from splenectomized rats by Noguchi (1927), Mayer and Kikuth (1927), Schilling (1929), &c.

Eperythrozoon coccoides.

During an investigation of the blood of splenectomized rats, Schilling (1928) met with another organism which also seems to attack the red cells when the spleen has been removed. It occurs in the cells, and also free in the plasma, in the form of small ring-like bodies, 1 to 2μ in diameter. These bodies also are especially obvious inside and on the surface of reticulated red cells. They have a reddish-violet colour with Giemsa's stain and the central area is almost unstained. When the infection is at its maximum very large numbers are found in the blood, and the polychromatophilic reticulocytes are often completely covered with them. If the animal survives, the parasites rapidly disappear from the blood about

6 to 8 days after splenectomy. For these parasites Schilling has suggested the name *Eperythrozoon coccoides*. They have been observed by other workers, and were seen by the writer in a mouse after splenectomy.

As has been already mentioned, however, there is still uncertainty as to whether such inclusions in the red cells of rats and mice are to be regarded as living parasites or merely as appearances associated with degeneration or immaturity of the cells. Of special importance as evidence of a living virus are the following observations: (1) washed red cells containing *Bartonella muris* are infective for animals rendered susceptible by splenectomy, whereas the corresponding plasma is either non-infective or only produces a very mild infection; (2) the agent producing both the anæmia and *Bartonella muris* is destroyed by heating at 57° C. for a short time (Ford and Eliot, 1928).

REFERENCES.

Barton, A. L., 1909, *Crón. méd.*, Lima, **26**, 7.

Bayon, H. P., 1928, *J. Trop. Med.*, **31**, 29.

Brumpt, E., 1911, *Bull. Soc. Path. exotique*, **4**, 514.

Cannon, P. R., Taliaferro, W. H. & Dragstedt, L. R., 1928, *Proc. Soc. Exp. Biol.*, N.Y., **25**, 359.

Da Cunha, A. M. & Muniz, J., 1927, *C.R. Soc. Biol.*, Paris, **97**, 1368.

Darling, S. T., 1911, *J. Amer. Med. Ass.*, **57**, 2071.

de Faria, G. & Cruz, O., Jr., 1927, *C.R. Soc. Biol.*, Paris, **96**, 489.

Ford, W. W. & Eliot, C. P., 1928, *J. Exp. Med.*, **48**, 475.

Gaillard, H. & Robles, R., 1928, *Ann. Parasit.*, **6**, 1.

Graham-Smith, G. S., 1905, *J. Hyg.*, Camb., **5**, 453.

Haan, E., Lauda, E. & Sorge, G., 1927, *Klin. Wschr.*, **6**, 2240.

Jadassohn, G. & Seiffert, G., 1910, *Z. Hyg. InfektKr.*, **66**, 247.

Jaffé, R. H. & Willis, D., 1928, *Proc. Soc. Exp. Biol.*, N.Y., **25**, 242.

Lauda, E., 1925, *Virchows Arch.*, **258**, 529; 1927, *Wien. med. Wschr.*, **77**, 772.

Lauda, E. & Marcus, F., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, Beiheft 194*.

Laveran, A. & Marullaz, M., 1914, *Bull. Soc. Path. exotique*, **7**, 240.

Mayer, M., 1921, *Arch. Schiffs- u. Tropenhyg.*, **25**, 150.

Mayer, M. & Kikuth, W., 1927, *Abh. Ausldsk.*, Hamburg Univ., **26** (D., Med. & Vet., Vol. 2), 319.

Mayer, M., Borchardt, W. & Kikuth, W., 1926, *Klin. Wschr.*, **5**, 559; 1927[1], *Deuts. med. Wschr.*, **53**, 9; 1927[2], *Arch. Schiffs- u. Tropenhyg.*, **31**, Beiheft 4.

Mayer, M., Rocha-Lima, H. & Werner, H., 1913, *Münch. med. Wschr.*, **60**, 739.

Nauck, E. G., 1927, *Arch. Schiffs- u. Tropenhyg.*, **31**, 322.

Noguchi, H., 1926, *J. Exp. Med.*, **44**, 697, 715 and 729; 1927, *ibid.*, **45**, 175, 437, 455 and 781; 1928, *ibid.*, **47**, 235.

Noguchi, H. & Battistini, T., 1926, *J. Exp. Med.*, **43**, 851.

Noguchi, H. & Hercelles, O., 1926, *Science*, n.s., **64**, 121.

Noguchi, H., Shannon, R. C., Tilden, E. B. & Tyler, J. R., 1929, *J. Exp. Med.*, **49**, 993.

Odriozola, E., 1896, *La maladie de Carrion*, Paris.

Schilling, V., 1928, *Klin. Wschr.*, **7**, 1853; 1929, *ibid.*, **8**, 55.

Strong, R. P., Tyzzer, E. E., Brues, C. T., Sellards, A. W. & Gastiaburu, J. C., 1915, *Report of 1st Expedition to S. America, 1913*, Harvard School of Trop. Med.

Townsend, C. H. T., 1913[1], *J. Amer. Med. Ass.*, **61**, 1717; 1913[2], *Peru To-day*, **5**, 840; 1927, *West Coast Leader*, March 8th.

Wenyon, C. M., 1926, *Protozoology*, London (Baillière, Tindall & Cox), **2**, 1058.

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